Published in final edited form as:

Hepatology. 2009 October ; 50(4): 1194–1203. doi:10.1002/hep.23120.

Hepatic Targeting and Biodistribution of Human Fetal Liver Stem/Progenitor Cells and Adult Hepatocytes in Mice

Kang Cheng^{1,*}, Daniel Benten^{1,*}, Kuldeep Bhargava², Mari Inada¹, Brigid Joseph¹, Christopher Palestro², and Sanjeev Gupta¹

¹ Marion Bessin Liver Research Center, Diabetes Research Center, and Cancer Research Center, Departments of Medicine and Pathology, Albert Einstein College of Medicine, Bronx, NY

² Division of Nuclear Medicine and Molecular Imaging, Long Island Jewish Medical Center, New York, NY

Abstract

Tracking stem/progenitor cells through noninvasive imaging is a helpful means of assessing the targeting of transplanted cells to specific organs. We performed in vitro and in vivo studies wherein adult human hepatocytes and human fetal liver stem/progenitor cells were labeled with indium-111 (¹¹¹In)-oxine and technetium-99m (^{99m}Tc)-Ultratag or ^{99m}Tc-Ceretec. The labeling efficiency and viability of cells was analyzed in vitro, and organ biodistribution of cells was analyzed in vivo after transplantation in xenotolerant nonobese diabetic/severe combined immunodeficiency mice through intrasplenic or intraportal routes. We found that adult hepatocytes and fetal liver stem/progenitor cells incorporated ¹¹¹In but not ^{99m}Tc labels. After radiolabeling, cell viability was unchanged. Transplanted adult hepatocytes or fetal liver stem/progenitor cells were targeted to the liver more effectively by the intraportal rather than the intrasplenic route. Transplanted cells were retained in the liver after intraportal injection and in the liver and spleen after intrasplenic injection, without translocations into pulmonary or systemic circulations. Compared with fetal liver stem/progenitor cells, fewer adult hepatocytes were retained in the spleen after intrasplenic transplantation. The distribution of transplanted cells in organs was substantiated by genetic assays, including polymerase chain reaction amplification of DNA sequences from a primate-specific Charcot-Marie-Tooth element, and in situ hybridization for primate alphoid satellite sequences ubiquitous in all centromeres.

Conclusion—¹¹¹In labeling of human fetal liver stem/progenitor cells and adult hepatocytes was effective for noninvasive localization of transplanted cells. This should facilitate continued development of cell therapies through further animal and clinical studies.

Numerous genetic and acquired disorders with or without a hepatic basis are amenable to liver-directed cell therapy.¹ The extensive re-generative capacity of the liver coupled with mechanisms regulating proliferation in transplanted cells facilitated cell therapy-based correction of disorders in animals, including when the liver is healthy (such as hemophilia A)² and when significant damage occurs in the liver (such as Wilson disease).³ Because the supply of human donor organs is limited, stem/progenitor cells are at the forefront of cell therapy applications. Although stem cells may be manipulated to derive organ-specific lineages in vitro, including liver-like cells,⁴ cell targeting and engraftment of transplanted cells in desired cell compartments are critical. However, these mechanisms are largely undefined.

Address reprint requests to: Sanjeev Gupta, M.D., Albert Einstein College of Medicine, Ullmann Building, Room 625, 1300 Morris Park Avenue, Bronx, NY 10461. sanjvgupta@pol.net; fax: 718-430-8975.

*These authors contributed equally to this work.

Potential conflict of interest: Nothing to report.

To a significant extent, the initial location of transplanted cells governs what fraction of cells will engraft, which in turn determines whether transplanted cells will repopulate an organ and whether organ repopulation will occur slowly or more rapidly.^{3,5} If transplanted cells were redistributed to certain vascular beds (such as pulmonary capillaries), vascular occlusion and embolic complications may result.^{6,7} Therefore, convenient and noninvasive means of determining the distribution of stem/progenitor cells will benefit cell therapy applications. Tissue sampling for assessing distributions of transplanted cells by way of histological or other assays is invasive and therefore less desirable. Imaging with extrinsic radiolabels such as indium-111 (¹¹¹In) and technetium-99m (^{99m}Tc) has been effective for studies of early cell biodistributions and cell fate in the hepatic or other vascular beds after transplantation of mature rodent hepatocytes or liver sinusoidal endothelial cells.^{6–9} Conjoint computed tomography, positron emission tomography, and magnetic resonance imaging offer complementary means of gathering more information about changes in organs.

To establish the initial fate of transplanted cells, we addressed the affinity of human fetal liver stem/progenitor cells and adult human hepatocytes for ¹¹¹In and ^{99m}Tc labels, followed by studies of cell biodistribution in xenograft-capable nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice. These studies were facilitated by previous characterization of adult hepatocytes isolated from donor human livers¹⁰ and of stem/progenitor cells isolated from cadaveric fetal livers.^{11,12} Fetal liver cells displayed the epithelial cell adhesion molecule, EpCAM, along with unique stem cell and multilineage markers; generated epithelial or mesenchymal lineages under suitable differentiation conditions; and proliferated extensively in culture, which was in agreement with their stem/progenitor properties. We found that human liver cells incorporated ¹¹¹In but not ^{99m}Tc. Moreover, we established that the mechanisms of cell bio-distribution in cases of radiolabeled adult hepatocytes or fetal liver stem/progenitor cells were broadly similar.

Materials and Methods

Human Fetal Liver Cells and Adult Hepatocytes

The Committee on Clinical Investigations at the Albert Einstein College of Medicine approved the use of human material. Fetal livers were obtained from the Human Fetal Tissue Repository of the Albert Einstein College of Medicine. Informed consent was obtained from donors before elective termination of 18- to 24-week pregnancies.

Fetal liver stem/progenitor cells were isolated and cryo-preserved as described.¹² Briefly, livers were digested with collagenase for 20 to 30 minutes at 37°C, and cells were passed through 80 μ m Dacron, pelleted under 350g for 5 minutes at 4°C, and resuspended in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA). For studies, frozen cells were rapidly thawed to 37°C. To isolate Ep-CAM-positive epithelial stem/progenitor cells, red blood cells were lysed in 155 mM NH₄Cl, 10 mM KHCO₃, and 0.1 mM ethylene diamine tetraacetic acid for 7 minutes on ice followed by immunomagnetic sorting (MACS; Miltenyi Biotec Inc., Bergisch Gladbach, Germany) as described.¹² EpCAM-positive and EpCAM-negative cell fractions were collected. Cell number and viability was determined using a Neubauer hemocytometer with exclusion of 0.2% trypan blue dye. Typically, $0.75\text{--}2 \times 10^8$ epithelial cells were isolated from each of the 18- to 24-week gestation livers. The viability of isolated fetal cells was 90% to 98%.

Adult hepatocytes were from ADMET Technologies Inc. (Durham, NC). These cells were isolated by way of collagenase digestion of donor livers (H0852-P10a, H0852-P15, and H0796-U10) followed by cryopreservation as described.¹⁰ The cell yield from adult donor livers is variable and depends on the underlying condition of the liver (that is, its fatty content). However, before cryo-preservation, viability of mature hepatocytes was 76% to 82%.

Cell Radiolabeling

EpCAM-positive, EpCAM-negative, mixed fetal stem/progenitor cells, and adult hepatocytes were incubated in normal saline with 150 MBq ^{111}In -oxine and 370–555 MBq $^{99\text{m}}\text{Tc}$ -UltraTag (Mallinckrodt Inc., Maryland Heights, MO) or $^{99\text{m}}\text{Tc}$ -Ceretek (Amersham Inc., Arlington Heights, IL), for 20 minutes at room temperature. $^{99\text{m}}\text{Tc}$ -UltraTag kit incorporates stannous ions, which enter cells and accumulate intracellularly, which in turn reduces $^{99\text{m}}\text{Tc}$ pertechnetate and traps Tc inside the cell. Similarly, a $^{99\text{m}}\text{Tc}$ -Ceretek kit incorporates stannous ions to form a lipophilic complex after $^{99\text{m}}\text{Tc}$ pertechnetate is added to exametazime. These kits are widely used for labeling red blood cells and white blood cells. We collected cells under 300g for 5 minutes and washed twice with cold phosphate-buffered saline (pH 7.4). To determine labeling efficiency, radioactivity was measured in cell pellets and pooled supernatants.

Cell Viability Assays

After labeling, cell viability was determined by way of trypan blue exclusion. Wet smears were prepared or cells were fixed in 4% paraformaldehyde in phosphate-buffered saline followed by hematoxylineosin staining for microscopic examination. In some studies, cells were cultured in Roswell Park Memorial Institute 1640 medium with 10% fetal bovine serum and antibiotics overnight followed by histochemical staining for glycogen or glucose-6-phosphatase, as described.¹⁰ To further demonstrate the effect of radiolabeling on hepatocyte function, we incubated ^{111}In -labeled human fetal stem/progenitor cells with 5 mM ammonium chloride in serum-free Roswell Park Memorial Institute 1640 medium for 2 hours at 37°C. This was followed by collection of medium for measurement of urea synthesis. We added 0.3 mL Urease Buffer Reagent (U-3383, Sigma) to 100 μL medium for 20 minutes at room temperature followed by 0.6 mL Phenol Nitroprusside Solution (P-6994, Sigma), 0.6 mL alkaline hypochlorite solution (A-1727, Sigma), and 3.0 mL deionized water, in that order, with mixing after each addition. The mixture was incubated for 30 minutes at room temperature, and absorbance at 540 nm was measured. Standards were prepared with 10 mg/mL urea (U-5128, Sigma).

Animals

The Animal Care and Use Committees at the Albert Einstein College of Medicine and Feinstein Institute for Medical Research approved the study protocols. NOD-CB17-prkdc-SCID mice, 6 to 8 weeks old, were obtained from the Jackson Laboratory (Bar Harbor, ME). Each mouse received 1×10^6 radiolabeled cells through the spleen or portal vein as described.^{9,13} We established eight groups of animals ($n = 3$ –6 each) for studies 1 hour after transplantation of unfractionated fetal cells, EpCAM-negative cells, EpCAM-positive cells, and adult hepatocytes. Two other groups of mice ($n = 3$ each) were established for studying engraftment 1 week after transplantation of EpCAM-positive fetal hepatocytes and adult hepatocytes. One hour after cell transplantation, total body images were acquired for 10 minutes in $64 \times 64 \times 16$ matrix at a zoom factor of 2 with a gamma camera (Argus; ADAC Laboratories, Milpitas, CA). Subsequently, mice were sacrificed and the liver, spleen, lungs, heart, and intestines were excised for measuring organ activity. Total radioactivity was used as a denominator for determining fractional distribution of activity in each of these organs. Other animals were sacrificed 1 week after cell transplantation to verify engraftment of cells. Tissue samples were frozen in methylbutane cooled to -80°C for cryosections.

Identification of Transplanted Human Cells in Mice

Genomic DNA was extracted with DNeasy Tissue Kit (Qiagen, Milpitas, CA). Polymerase chain reaction (PCR) was for Charcot-Marie-Tooth disease type 1A repeat element (CMT1A) as described^{14,15} (forward primer, 5'-GAGTGCACATTCAGACAAGAGCCC-3'; reverse

primer, 5'-CCATTAGAGAGCTTTCCTCATTGC-3'). The integrity of complementary DNAs was verified by amplifying the glyceraldehyde 3-phosphate dehydrogenase gene with forward primer 5'-CCATGGAGAAGGCT-GGGG-3' and reverse primer 5'-CAAAGTTGTCAT-GGATGACC-3'. PCR was performed for 40 cycles with denaturation at 95°C × 30 seconds, annealing at 55°C × 30 seconds, and extension × 1 minute. PCR products were resolved in 1.8% agarose gels containing ethidium bromide.

The presence of transplanted cells was verified by way of in situ hybridization with a primate pancentromeric alphoid satellite probe as described.¹⁴ Hybridization signals were visualized through the incubation of tissue cryo-sections for 1.5 hours at 37°C in 80 to 100 µL peroxidase-conjugated or fluorescein isothiocyanate-conjugated anti-digoxigenin (1:50; Roche Life Sciences, Indianapolis, IN). Color was developed over 20 minutes with diaminobenzidine. Counterstaining was with toluidine blue or 4', 6-diamidino-2-phenylindole.

Statistical Analysis

Data are expressed as the mean ± standard deviation. Significance was determined using the Student *t* test or analysis of variance with SigmaStat 3.1 (Systat Software Inc., Point Richmond, CA); *P* < 0.05 was considered significant.

Results

Human Fetal and Adult Liver Cells Incorporated Only ¹¹¹In

We used separate donor livers to demonstrate whether human cells will incorporate ¹¹¹In and ^{99m}Tc. Adult human hepatocytes efficiently incorporated ¹¹¹In to 78% ± 2% (Table 1). Also, fetal liver cells incorporated ¹¹¹In, although the labeling efficiency varied (51% ± 9% in unfractionated fetal liver cells; 36% ± 9% in EpCAM-positive cells; 62% ± 11% in EpCAM-negative cells [*P* < 0.05 versus adult hepatocytes (analysis of variance)]). Neither adult hepatocytes nor fetal liver cells incorporated ^{99m}Tc labels, suggesting fundamental differences in the uptake of ¹¹¹In versus ^{99m}Tc in cells.

Radiolabeling did not change viability with trypan blue exclusion studies in either fetal cells or adult hepatocytes. The viability after radiolabeling of fetal cells was 98% ± 3% and of adult hepatocytes was 94% ± 7% of respective unlabeled controls that were incubated under identical conditions in vitro. Labeled cells were intact and showed unchanged morphology (Fig. 1). Unfractionated fetal liver cells were a mixture of small and large nucleated cells. EpCAM-negative cells were depleted of large epithelial cells. EpCAM-positive cells were all large epithelial cells. Adult human hepatocytes were characteristically large and round.

In addition to demonstrating intact morphology, after radiolabeling, cells attached to culture plastic and exhibited normal content of glycogen and glucose-6-phosphatase, which was consistent with their functional integrity. Moreover, when cells were incubated under identical conditions with or without ¹¹¹In followed by incubation with ammonium chloride to assess synthetic function (ureagenesis), we found that radiolabeled hepatocytes were functionally intact (Fig. 2).

¹¹¹In-Labeled Human Cells Were Mostly in the Liver in NOD/SCID Mice

In animals, where fetal liver cells were transplanted via the spleen, imaging showed transplanted cells in the spleen as well as the liver (Fig. 3). The distribution of transplanted cells was the same irrespective of whether fetal liver cells were unfractionated, EpCAM-positive, or EpCAM-negative. By contrast, when fetal liver cells were transplanted by way of the portal vein, transplanted cells were visualized only in the liver. Transplantation of adult hepatocytes resulted in visualization of the spleen and liver after intrasplenic transplantation

and only the liver after intraportal transplantation. Transplanted cells were not visualized outside the liver or spleen in other organs, indicating an absence of cell translocations elsewhere in substantive numbers.

Analysis of excised organs for transplanted cells verified that more radioactivity was in the liver after intraportal injection versus after intrasplenic injection of fetal cells under various conditions (means of 93% to 97% versus 52% to 59%, respectively; $P < 0.05$ [analysis of variance]) (Fig. 4). This difference was due to entrapment of intrasplenically transplanted cells in the spleen, which ranged from 38% to 40%. After intraportal transplantation of unfractionated fetal cells, slightly more cells were distributed to the lungs compared with EpCAM-negative or EpCAM-positive cells. By contrast, adult hepatocytes were retained less in the spleen, and distributions of adult hepatocytes in organs after intrasplenic or intraportal transplantation were similar.

Genetic Assays Verified Presence of Human Cells in Mice

We detected CMT1A sequences by way of PCR in the liver and spleen when cells were injected into the spleen (Fig. 5). By contrast, when cells were injected into the portal vein, CMT1A sequences were found only in the liver. CMT1A sequences were not detected in the lungs, heart, kidneys, or intestine, indicating that transplanted human cells either did not enter these organs or did so rarely, below the threshold of detection. Moreover, CMT1A sequences were detected in the liver after 1 week, indicating that radiolabeling did not prevent long-term engraftment of fetal or adult human cells. These data concerning the presence of transplanted cells in various organs were in agreement with the organ biodistribution studies described above.

In situ hybridization verified that transplanted cells were present in the liver and spleen. The pancentromeric human probe produced multiple signals in the nuclei of human cells and not in mouse cells, as expected (Fig. 6). This permitted unequivocal identification of transplanted fetal human cells, which were in vascular spaces of the liver and spleen 1 hour after transplantation (Fig. 6C, D). We observed one to three transplanted cells in or adjacent to individual portal areas, which was expected from previous cell transplantation studies in mice.^{12,13}

Similarly, transplanted adult human hepatocytes were distributed in vascular spaces initially, whereas after 1 week, transplanted cells were found in the liver parenchyma after transplantation by way of the spleen (Fig. 7C) or portal vein (Fig. 7D), indicating that transplanted cells engrafted and integrated in the liver structure.¹²

Overall, in situ hybridization studies 1 hour and 1 week after intrasplenic cell transplantation showed transplanted cells in the liver and spleen, irrespective of whether unfractionated, EpCAM-negative, or EpCAM-positive fetal cells or adult hepatocytes had been transplanted. By contrast, after intraportal cell transplantation, transplanted fetal or adult cells were localized in only the liver. Transplantation of nonradiolabeled cells in the spleen resulted in similar findings of cell engraftment with occasional human cells in the spleen and one to three transplanted cells in the liver lobule adjacent to portal areas (data not shown).

Discussion

We have demonstrated that adult human hepatocytes and human fetal liver stem/progenitor cells incorporate ¹¹¹In and not ^{99m}Tc labels; that intraportal transplantation of cells is more effective than intrasplenic transplantation for delivering cells to the liver; that transplanted cells are largely restricted to the liver without onward exit to pulmonary or systemic circulations; and that biodistribution of fetal stem/progenitor cells and adult hepatocytes is mostly similar.

These observations will be helpful in assessing the potential of candidate stem/progenitor cell populations for cell therapy applications, as well as for monitoring the fate of transplanted stem/progenitor cells in clinical studies.

^{111}In is an inert metal that is not normally encountered in biological life systems, although complexing with oxine renders it neutral and lipid-soluble, permitting transfer across the cell membrane, followed by binding to cytosolic proteins. This characteristic may account for its successful incorporation in adult hepatocytes and fetal liver stem/progenitor cells, including their epithelial and non-epithelial cell fractions. We established that ^{111}In labeling did not alter cell viability or function, as indicated by the urea synthesis assay. This finding was similar to those of studies in rodent hepatocytes, which additionally showed by autoradiography that the incorporated ^{111}In was inside cells in the cytoplasm.⁸ Moreover, radiolabeling did not perturb engraftment of transplanted cells in the mouse liver over the 1-week duration of our studies. It is noteworthy that cell engraftment is completed within that period, and that engrafted cells survive indefinitely afterward.¹⁶ Because our studies were restricted to transplantation of cells in small numbers, it was not feasible to demonstrate the release of secreted human proteins (such as albumin in the blood of recipient mice), and induction of proliferation in transplanted cells or disease correction with cell transplantation would have required extended studies in alternative animal models. However, such studies should be unnecessary, because ample evidence exists to indicate that when transplanted hepatocytes integrate in the liver parenchyma, normal hepatic functions are maintained under a variety of conditions, including in disease models.^{2,3,6,17}

Neither human fetal liver cells nor adult human hepatocytes incorporated $^{99\text{m}}\text{Tc}$ -Ultratag or $^{99\text{m}}\text{Tc}$ -CereteC. This was unexpected on at least two counts. First, rodent hepatocytes were efficiently labeled with $^{99\text{m}}\text{Tc}$ -Ultratag or $^{99\text{m}}\text{Tc}$ -CereteC according to the conditions used herein, and this was successful for biodistribution studies.⁷ Second, red blood cells and peripheral blood leukocytes show an excellent affinity for $^{99\text{m}}\text{Tc}$ -Ultratag and $^{99\text{m}}\text{Tc}$ -CereteC, respectively.^{18,19} Because midgestation fetal human liver has an abundance of nucleated hematopoietic cells due to its role in extramedullary hematopoiesis,¹¹ the inability of unfractionated fetal liver cells to incorporate $^{99\text{m}}\text{Tc}$ suggests fundamental differences in mature versus immature cells. Although precise mechanisms in cellular uptake of $^{99\text{m}}\text{Tc}$ are undefined, such a difference should reinforce the need for systematic analysis of molecular imaging ligands and substrates for applications in stem/progenitor cells and stem cell-derived cells.

Our studies demonstrated that intraportal rather than intrasplenic delivery was more effective for depositing stem/progenitor cells in the liver. For some studies, the presence of cells in the spleen could be beneficial (for example, to assess context-specific regulation of cell differentiation, survival, or proliferation).²⁰ In addition, deposition of cells in the spleen has been of some therapeutic interest (for example, for creating an extrahepatic reservoir of transplanted hepatocytes).²¹ After injection into the splenic pulp, most hepatocytes immediately translocate to splenic veins, followed by the portal vein, and then to hepatic sinusoids, although hepatocytes trapped in splenic sinusoids may engraft in the spleen itself.^{8,20} On the other hand, the entry of cells in pulmonary capillaries is a concern, because this may produce embolic complications.^{7,8} Therefore, tracking of transplanted cells will be helpful in demonstrating deleterious distributions of cells. Although the ability to track transplanted cells in an indefinite manner will be ideal, especially for clinical applications, the half-life of ^{111}In is relatively short—2.8 days, which restricts imaging to the short-term. Moreover, macrophages and phagocytes clear substantial fractions of transplanted cells from the liver sinusoids,⁵ inevitably leading to secondary radiotracer uptake. It was noteworthy that a biodistribution analysis of adult hepatocytes, as well as epithelial and non-epithelial fractions of fetal liver stem/progenitor cells, indicated that transplanted cells were restricted to the liver and spleen. This should not be surprising, because epithelial liver cells are larger (15–30 μm)

than nucleated hematopoietic and other cells (8–12 μm), hepatic sinusoids are much smaller (3–6 μm). The size–structure relationships between transplanted cells and hepatic sinusoids indicated that human liver cells will enter more distal locations of the liver lobule,²² although without necessarily exiting from the healthy liver. However, in portal hypertension and/or portosystemic shunting, cell biodistributions would be different.⁶ This latter circumstance will constitute an appropriate clinical setting for prospective studies of transplanted cell distributions. Whether nonepithelial liver cells were targeted to additional vascular beds through adhesion molecule–based or cell surface receptor–based interactions is not excluded. For instance, previous studies established that transplanted hepatocytes were targeted to native liver sinusoidal endothelial cells through interactions involving integrin and extracellular matrix components.²³ Other molecules, such as stromal cell-derived factor-1, helped in the targeting of hematopoietic stem cells.²⁴

In case of fetal and adult human liver cells, our biodistribution analysis of organs verified restriction of transplanted cells to the liver and spleen as shown by imaging studies. Moreover, those data showed good correlation with molecular assays, including PCR and in situ hybridization studies. Previously, other human-specific PCR probes (sex-determining region Y, repetitive intronic Alu sequences, and other methods) were successful for identifying human cells in chimeric mice.^{10,15} Tissue sampling poses obvious restrictions in clinical situations involving people or large animals, such as primates. In recent detailed studies, we examined the sensitivity of quantitative real-time polymerase chain reactions for genetic probes to identify transplanted human cells in chimeric mice.¹⁵ These human-specific probes included the Charcot-Marie-Tooth disease, type 1 repeat element, sex-determining region Y, or short tandem repeats across human leukocyte antigen regions. None of the probes could identify transplanted human cells constituting less than 1% of the total cell mass in the mouse liver. Therefore, radiolabeling of cells will not only permit noninvasive imaging for assessing biodistributions of transplanted cells, it will be far more sensitive than genetic probes requiring tissue sampling. Also, it should be noteworthy that not all transplanted cells need to be radiolabeled, because a mixture of radiolabeled and nonradiolabeled cells should be equally effective.

Our methods to radiolabel rodent hepatocytes were successfully applied for tracking transplanted adult hepatocytes in a child.²⁵ Similarly, ¹¹¹In-based imaging should be effective for studies in small and large animals and in people to develop therapies with human stem cells. The widespread availability and routine use of ¹¹¹In in nuclear medicine should be a particular advantage, although ^{99m}Tc is equally in routine clinical use and could have provided the advantage of better quality gamma images.

Acknowledgments

We thank Mark Johnston (AD-MET Technologies, Inc.) for providing adult human hepatocytes.

Supported in part by National Institutes of Health Grants R01 DK46952, R01 DK071111, and P30 DK41296 (to S. G.) and by a grant from the State of New York for stem cell research (to the Albert Einstein College of Medicine). D. B. was supported in part by a scholarship from the German Research Foundation (BE-2559/1-1).

Abbreviations

¹¹¹ In	indium-111
NOD/SCID	nonobese diabetic/severe combined immunodeficiency
PCR	polymerase chain reaction
^{99m} Tc	technetium-99m

References

1. Fisher RA, Strom SC. Human hepatocyte transplantation: worldwide results. *Transplantation* 2006;82:441–449. [PubMed: 16926585]
2. Follenzi A, Benten D, Novikoff P, Faulkner L, Raut S, Gupta S. Transplanted endothelial cells repopulate the liver endothelium and correct the phenotype of hemophilia A mice. *J Clin Invest* 2008;118:935–945. [PubMed: 18274668]
3. Joseph B, Kapoor S, Schilsky ML, Gupta S. Hydrophobic bile salt-induced perturbations and proliferation of transplanted cells in the liver of rats with copper toxicosis. *HEPATOLOGY* 2009;49:1616–1624. [PubMed: 19185006]
4. Basma H, Soto-Gutiérrez A, Yannam GR, Liu L, Ito R, Yamamoto T, et al. Differentiation and transplantation of human embryonic stem cell-derived hepatocytes. *Gastroenterology* 2009;136:990–999. [PubMed: 19026649]
5. Joseph B, Malhi H, Bhargava KK, Palestro CJ, McCuskey RS, Gupta S. Kupffer cells participate in early clearance of syngeneic hepatocytes transplanted in the rat liver. *Gastroenterology* 2002;123:1677–1685. [PubMed: 12404242]
6. Gupta S, Yerneni P, Vemuru PP, Lee CD, Yellin EL, Bhargava KK. Studies on the safety of intrasplenic hepatocyte transplantation: relevance to ex vivo gene therapy and liver repopulation in acute hepatic failure. *Hum Gene Ther* 1993;4:249–257. [PubMed: 8338873]
7. Rajvanshi P, Fabrega A, Bhargava KK, Kerr A, Pollack G, Blanchard J, et al. Rapid clearance of hepatocytes from pulmonary capillaries in rats allows development of surrogates for testing safety of liver repopulation. *J Hepatol* 1999;30:299–310. [PubMed: 10068111]
8. Gupta S, Lee CD, Vemuru RP, Bhargava KK. ¹¹¹Indium labeling of hepatocytes for analysis of short-term biodistribution of transplanted cells. *HEPATOLOGY* 1994;19:750–757. [PubMed: 8119703]
9. Benten D, Follenzi A, Bhargava KK, Kumaran V, Palestro CJ, Gupta S. Organ-specific targeting of transplanted endothelial cells in intact mice. *HEPATOLOGY* 2005;42:140–148. [PubMed: 15918158]
10. Cho JJ, Joseph B, Sappal BS, Giri RK, Wang R, Ludlow JW, et al. Analysis of the functional integrity of cryopreserved human liver cells including xenografting in immunodeficient mice to address suitability for clinical applications. *Liver Int* 2004;24:361–370. [PubMed: 15287860]
11. Inada M, Benten D, Cheng K, Joseph B, Berishvili E, Badve S, et al. Stage-specific regulation of adhesion molecule expression segregates epithelial stem/progenitor cells in fetal and adult human livers. *Hepatol Int* 2008;2:50–62. [PubMed: 19669279]
12. Inada M, Follenzi A, Cheng K, Surana M, Joseph B, Benten D, et al. Phenotype reversion in fetal human liver epithelial cells identifies the role of an intermediate mesoendodermal stage before hepatic maturation. *J Cell Sci* 2008;121:1002–1013. [PubMed: 18319302]
13. Wu YM, Joseph B, Berishvili E, Kumaran V, Gupta S. Hepatocyte transplantation and drug-induced perturbations in liver cell compartments. *HEPATOLOGY* 2008;47:279–287. [PubMed: 17935178]
14. Benten D, Cheng K, Gupta S. Identification of transplanted human cells in animal tissues. *Methods Mol Biol* 2006;326:189–201. [PubMed: 16780202]
15. Cheng K, Gupta S. Quantitative tools for assessing fate of transplanted human stem/progenitor cells in chimeric mice. *Xenotransplantation* 2009;16:145–151. [PubMed: 19566654]
16. Sokhi RP, Rajvanshi P, Gupta S. Transplanted reporter cells help in defining onset of hepatocyte proliferation during the life of F344 rats. *Am J Physiol Gastroint Liver Physiol* 2000;279:G631–G640.
17. Gupta S, Rajvanshi P, Sokhi R, Vaidya S, Irani AN, Gorla GR. Position-specific gene expression in the liver lobule is directed by the microenvironment and not by the previous cell differentiation state. *J Biol Chem* 1999;274:2157–2165. [PubMed: 9890978]
18. Rehani MM, Sharma SK. Site of Tc-99m binding to the red blood cell: concise communication. *J Nucl Med* 1980;21:676–678. [PubMed: 7391843]
19. Mesquita CT, Correa PL, Felix RC, Azevedo JC, Alves S, Oliveira CC, et al. Autologous bone marrow mononuclear cells labeled with Tc-99m hexamethylpropylene amine oxime scintigraphy after intracoronary stem cell therapy in acute myocardial infarction. *J Nucl Cardiol* 2005;12:610–612. [PubMed: 16171723]

20. Gupta S, Vemuru RP, Lee CD, Yerneni PR, Aragona E, Burk RD. Hepatocytes exhibit superior transgene expression after transplantation into liver and spleen compared with peritoneal cavity or dorsal fat pad: implications for hepatic gene therapy. *Hum Gene Ther* 1994;5:959–967. [PubMed: 7948145]
21. Nagata H, Ito M, Cai J, Edge AS, Platt JL, Fox IJ. Treatment of cirrhosis and liver failure in rats by hepatocyte xenotransplantation. *Gastroenterology* 2003;124:422–431. [PubMed: 12557148]
22. Rajvanshi P, Kerr A, Bhargava KK, Burk RD, Gupta S. Studies of liver repopulation using the dipeptidyl peptidase IV deficient rat and other rodent recipients: cell size and structure relationships regulate capacity for increased transplanted hepatocyte mass in the liver lobule. *HEPATOLOGY* 1996;23:482–496. [PubMed: 8617428]
23. Kumaran V, Joseph B, Benten D, Gupta S. Integrin and extracellular matrix interactions regulate engraftment of transplanted hepatocytes in the rat liver. *Gastroenterology* 2005;129:1643–1653. [PubMed: 16285962]
24. Czarnowska E, Gajerska-Dzieciatkowska M, Kuśmierski K, Lichomski J, Machaj EK, Pojda Z, et al. Expression of SDF-1-CXCR4 axis and an anti-remodelling effectiveness of foetal-liver stem cell transplantation in the infarcted rat heart. *J Physiol Pharmacol* 2007;58:729–744. [PubMed: 18195484]
25. Bohnen NI, Charron M, Reyes J, Rubinstein W, Strom SC, Swanson D, et al. Use of indium-111-labeled hepatocytes to determine the biodistribution of transplanted hepatocytes through portal vein infusion. *Clin Nucl Med* 2000;25:447–50. [PubMed: 10836694]

Fig. 1. Integrity of radiolabeled cells. Shown are representative images of EpCAM-positive fetal liver cells under phase contrast (left) or after hematoxylineosin staining (right). Cells were intact following incubation (A) without or (B) with ^{111}In (magnification $\times 400$).

Fig. 2. Maintenance of hepatic function after radiolabeling. Shown are data from fetal human liver stem/progenitor cells without or after ^{111}In labeling followed by analysis of urea synthesis. This hepatic synthetic function was maintained in radiolabeled cells. The data were obtained under triplicate conditions.

Fig. 3. Distribution of radiolabeled human cells in mice. Shown are images of mice after transplantation of ^{111}In labeled cells through the spleen (left) or portal vein (right). These images indicate distribution of (A) unfractionated mixed cells, (B) EpCAM-negative cells, (C) EpCAM-positive cells, and (D) adult hepatocytes. Radiolabeled cells were largely in the liver and spleen after intrasplenic injection, whereas after intra-portal injection, cells were largely in the liver.

Fig. 4.

Biodistribution of ^{111}In -labeled human liver cells in specific organs. Shown is distribution of radiolabeled cells in the liver, spleen, lungs, heart, and intestine 1 hour after intrasplenic or intraportal transplantation of cells in mice ($n = 3$ each). Animals received fetal liver cells that were (A) unfractionated, (B) EpCAM-negative, or (C) EpCAM-positive or (D) adult hepatocytes. The mean fractions of transplanted cells within the organs indicated are provided above the bars. A significant amount of ^{111}In activity was retained in the spleen after intrasplenic transplantation of fetal cells, which were better targeted to the liver by intraportal cell transplantation. By contrast, adult hepatocytes were retained less in the spleen, and distributions of adult hepatocytes after intrasplenic or intraportal transplantation were similar.

* $P < 0.05$ versus data in corresponding bars.

Fig. 5.

Detection of CMT1A human sequences by way of PCR in mouse tissues. Shown are data from recipients of (A, B) fetal EpCAM-positive cells or (C, D) adult hepatocytes 1 hour after intrasplenic or intraportal transplantation (A, C, D) as well as 1 week after intraportal transplantation (B). After intrasplenic cell transplantation, CMT1A sequences were detected in the liver and spleen (A, C). After intraportal cell transplantation, CMT1A sequences were detected in only the liver (B, D), including after 1 week (B). Mouse glyceraldehyde 3-phosphate dehydrogenase verified the integrity of complementary DNAs.

Fig. 6.

In situ hybridization to localize fetal human cells in mice. Shown are studies with digoxigenin-labeled pancentromeric human probe visualized after hybridization by epifluorescence. (A) Human liver with 4'6-diamidino-2-phenylindole staining alone (left), fluorescent in situ hybridization signals (middle), and merged image (right). (B) Nonreactive mouse liver without hybridization signals. (C, D) EpCAM-positive fetal human liver cells (arrows) in the spleen and liver, respectively, 1 hour after intrasplenic (C) or intraportal (D) transplantation (magnification $\times 400$). PA, portal area.

Fig. 7.

In situ hybridization for adult human hepatocytes in mice. Shows visualization of pancentromeric human probe signals by light microscopy. (A) Brown signals of in situ hybridization with diaminobenzidine in positive control human liver. (B) Absence of signals in negative control mouse liver. (C, D) Human hepatocytes (arrows) 1 week after intrasplenic (C) or intraportal (D) transplantation. The insets in panels C and D show magnified views of transplanted cells integrated in liver parenchyma (magnification $\times 400$). PA, portal area

Table 1

Radiolabeling Characteristics of Human Liver Cells

Cells Labeled	Labeling Efficiency (%)		
	¹¹¹ In	^{99m} Tc-Ultratag	^{99m} Tc-Ceretec
Unfractionated fetal liver cells	51 ± 9 (n = 5)	0 ± 0 (n = 3)	0 ± 0 (n = 2)
EpCAM-negative fetal liver cells	62 ± 11 (n = 3)	ND	ND
EpCAM-positive fetal liver cells	38 ± 9 (n = 3)	ND	ND
Adult human hepatocytes	78 ± 2 (n = 3)	0 ± 0 (n = 3)	0 ± 0 (n = 3)

Abbreviation: ND, not done.