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Repopulation Efficiencies of Adult Hepatocytes, Fetal Liver Progenitor Cells, and Embryonic Stem Cell-Derived Hepatic Cells in Albumin-Promoter-Enhancer Urokinase-Type Plasminogen Activator Mice

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Fetal liver progenitor cell suspensions (FLPC) and hepatic precursor cells derived from embryonic stem cells (ES-HPC) represent a potential source for liver cell therapy. However, the relative capacity of these cell types to engraft and repopulate a recipient liver compared with adult hepatocytes (HC) has not been comprehensively assessed. We transplanted mouse and human HC, FLPC, and ES-HPC into a new immunodeficient mouse strain (Alb-uPA$^{tg(+/-)}$Rag2$^{(-/-)}\gamma_c^{(-/-)}$ mice) and estimated the percentages of HC after 3 months. Adult mouse HC repopulated approximately half of the liver mass (46.6 ± 8.0%, 1 × 10^6 transplanted cells), whereas mouse FLPC derived from day 13.5 and 11.5 post conception embryos generated only 12.1 ± 3.0% and 5.1 ± 1.1%, respectively, of the recipient liver and smaller cell clusters. Adult human HC and FLPC generated overall less liver tissue than mouse cells and repopulated 10.0 ± 3.9% and 2.7 ± 1.1% of the recipient livers, respectively. Mouse and human ES-HPC did not generate HC clusters in our animal model. We conclude that, in contrast to expectations, adult HC of human and mouse origin generate liver tissue more efficiently than cells derived from fetal tissue or embryonic stem cells in a highly immunodeficient Alb-uPA transgenic mouse model system. These results have important implications in the context of selecting the optimal strategy for human liver cell therapies. ***(Am J Pathol 2009, 175:1483–1492; DOI: 10.2353/ajpath.2009.090117)***

Transplanted adult hepatocytes (HC) engraft in a recipient liver and morphologically as well as functionally connect with the surrounding cells.[1,2] In animal models with liver injury and/or selective growth advantage engrafted cells respond to growth stimuli and repopulate recipient livers.[3,4] Elucidation of the molecular pathways of liver regeneration and extensive preclinical cell transplantation experiments in animals have led to the application of HC transplantation in a limited number of patients with hereditary metabolic liver disease and acute liver failure.[5–8] However, the shortage of donor organs and the difficulties of cryopreservation and long-term culturing of

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mature HC have limited the clinical application of cell-based therapies.

Stem cells have attracted considerable interest for cell replacement therapy, because they expand in cell culture or can be easily harvested from patients.[9,10] Adult, fetal, and embryonic stem cell (ESC) sources have been studied as a potential substitute for primary adult HC in liver cell therapy. The generation of HC has been reported in recipient livers of animals, which have been transplanted with adult hematopoietic and mesenchymal stem cells.[11–14] More recent studies, however, have not convincingly shown formation of HC in therapeutically relevant numbers in mouse liver repopulation or toxic injury models.[15–17] In one study in fumarylacetoacetate hydrolase (Fah)$^{(-/-)}$ deficient mice, liver tissue formation from transplanted bone marrow cells was found to be the result of monocyte fusion with recipient liver cells.[18] In contrast to adult stem cells, fetal liver progenitor cells (FLPC) already express an induced immature hepatic phenotype and can be isolated, cultured, and expanded *in vitro*. Transplantation experiments in several laboratories have demonstrated engraftment of FLPC and subsequent liver tissue formation.[19,20] Transplanted FLPC, which were isolated from murine fetal liver tissue, were shown to acquire the adult HC phenotype over a period of 6 to 8 weeks after transplantation.[21] Although FLPC can be expanded in cell culture, the availability of donated fetal tissues restricts the clinical application of this cell source.

With their unlimited potential to grow *in vitro* and to develop into virtually any cell type, ESCs, and more recently, induced pluripotent stem cells, might be the ideal source of donor liver cells for cell therapies in the future.[22–24] We and others have generated hepatic precursor cells from human and mouse ESC lines.[25–28] With the existing differentiation protocols a primitive hepatic phenotype with fetal gene expression patterns can be induced in the majority of the ESCs.[28,29] Transplantation of these cells, however, have so far resulted only in scattered formation of HC or were reported to form small HC clusters in major urinary protein promoter driven urokinase-type plasminogen activator (uPA) mice[30] and Fah$^{(-/-)}$ mice.[31]

Multiple progenitor cell types have been studied extensively in transplantation experiments in animals with normal liver, in toxic liver injury models, and in liver repopulation models such as the albumin promoter/enhancer (Alb) directed uPA transgenic or Fah$^{(-/-)}$ mice. Although the potential of transplanted stem cell derived hepatic precursor and progenitor cells to generate HC has been clearly demonstrated, a comparative analysis of the individual capacity to form liver tissue is not available. In our present study we aimed to establish and validate an animal model, which would allow us to compare side-by-side the degree of liver repopulation of various human and murine cell types in a recipient liver. To this end, we performed standardized transplantation experiments in immunodeficient heterozygous Alb-uPA mice. In this animal model the transgene is expressed under transcriptional control of the albumin promoter/enhancer sequence exclusively in HC, which causes postnatal toxic liver injury.[32] Homozygous mice die from liver failure, but can be rescued by the transplantation of HC. In heterozygous mice, endogenous HC delete the transgene and regenerate the liver. Transplanted cells thus compete with endogenous HC to regenerate the liver. The capacity of a given cell type to repopulate a recipient liver organ after transplantation in this animal model is determined by its engraftment properties, the *in vivo* differentiation potential, and the proliferation capacity in a recipient liver. We generated a new immunodeficient xenograft mouse model by crossing Alb-uPA transgenic (tg) mice onto the Rag2$^{(-/-)}\gamma_c^{(-/-)}$ background (Alb-uPA$^{tg(+/-)}$Rag2$^{(-/-)}\gamma_c^{(-/-)}$ mice). This new model was then transplanted with various primary human and mouse cells with hepatic phenotype and liver tissues of the transplanted animals were harvested 3 months after transplantation and analyzed for the presence of HC derived from transplanted cells. Our data indicate that immature hepatic cell types of both human and mouse origin are unexpectedly less competitive compared with adult HC in repopulation of the Alb-uPA$^{tg(+/-)}$Rag2$^{(-/-)}\gamma_c^{(-/-)}$ mouse liver. Additionally, the overall repopulation rates observed after transplantation of human fetal and adult cells were significantly lower compared with similar transplantations performed with respective mouse cells.

Materials and Methods

Animals

C57BL/6 and enhanced green fluorescent protein (EGFP)-transgenic mice (C57BL/6-TgN(ACTbEGFP)1Osb) were purchased from the Jackson Laboratory (Bar Harbor, ME). Alb-uPA$^{tg(+/-)}$Rag2$^{(-/-)}\gamma_c^{(-/-)}$ mice were generated by breeding of Alb-uPA transgenic mice[32,33] on the severe combined immunodeficiency background[34] with Rag2$^{(-/-)}\gamma_c^{(-/-)}$ mice[35] on the nonobese diabetic background (J.P. Di Santo, unpublished). All animals were maintained and handled in accordance with institutional guidelines of the Hannover Medical School and the Helmholtz Center for Infection Research.

Isolation of Cells from Human Adult and Fetal Liver Tissue

Human adult HC were isolated as described previously by a modified three-step collagenase perfusion from surgical resectates, which have been obtained from patients with informed consent.[36] Perfusion solutions were introduced into the tissue through catheters placed into the portal or hepatic vein branches. After the digestion phase, the liver tissue was manually disrupted with sterile scissors and scalpels. To separate undigested tissue pieces, the suspended HC were passed through 750 and 500 μm filters into 50 ml Falcon tubes. The cell suspensions were centrifuged at 50 g for 10 minutes and the cell pellet was resuspended in an ice cold buffer. An aliquot of the cell preparation was separated for cell count and viability analysis (light microscopy and trypan blue exclusion test). In all transplantation experiments, suspensions