ELSEVIER

cMet Activation Allows Persistent Engraftment of Ectopically Transplanted Xenogenic Human Hepatocytes in Mice

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HEPATOCYTE TRANSPLANTATION (HT) is considered a therapeutic alternative to organ transplantation for end-stage and enzyme-deficient liver diseases.[1] Among several early clinical trials of HT, one has shown evidence of therapeutic benefit in a patient with Crigler-Najar syndrome.[2] In current trials, the number of hepatocytes that were infused through the portal circulation was limited to 5% of the total liver mass to minimize potential complications related to the cell infusion. The ectopic transplantation (eg, under the kidney capsule or subcutaneous space) of hepatocytes has a decreased potential for complications observed with intraportal infusion and allows for more space to transplant a greater number of donor cells.

An important factor required for achieving successful sustained and functional exogenous HT is to maintain the supply of hepatotrophic stimulation. Some studies have successfully provided a proliferation stimulus to hepatocytes by performing complicated procedures, such as portacaval shunting or inducing liver injury by adenoviral-mediated gene transfer to the liver.[3,4] These methods severely limit their current clinical applications. Here we describe the use of a human cMET agonistic antibody to obtain persistent engraftment of the human hepatocytes transplanted into two different ectopic sites in immunodeficient mice. We demonstrate that one of the mechanisms of hepatocellular persistence is related to hepatocellular proliferation in vivo.

MATERIALS AND METHODS

Human hepatocytes were isolated from the liver piece excised at the liver surgery using modified two-step collagenase perfusion method[5] and were purified by three rounds of low-speed centrifugation. Isolated hepatocytes were resuspended in William's E medium with an equal volume of cold liquid Matrigel (Becton Dickinson, Franklin Lakes, NJ). Female NOD/SCID mice, 6 to 8 weeks old, were used as a recipient. A total of 4×10^6 hepatocytes in 0.4-mL suspension were transplanted under kidney capsule space (KC group) or 6×10^6 hepatocytes in 0.6-mL suspension were transplanted in subcutaneous space on the back (SC group). Each group was divided into two subgroups; one received the c-Met antibody described below, and the other received carrier solution, saline.[6] Monoclonal human c-Met antibody was produced by immunizing mouse with a recombinant form of the extracellular domain of human c-Met. This c-Met antibody induces tyrosine phosphorylation in human-derived cells but not in mouse cells. Fifty micrograms of the antibody was resuspended in 250 μL of saline and was administered intravenously every 2 weeks for the first 57 days in c-Met group.

Viability and maintenance of the ectopically transplanted human hepatocytes in vivo were determined by periodical measuring a human hepatocyte specific serum marker, alpha-1 antitrypsin (hAAT), as described previously.[7] In some mice an osmotic minipump (Alze, Palo Alto, Calif) was placed subcutaneously to administer BrdU (1 mg/d) for 2 weeks after the transplantation.

RESULTS

The survival of the transplanted human hepatocytes measured by serum hAAT levels in both KC and SC groups slowly declined over a period of 3 months. Despite the fact that the KC group received a small number of hepatocytes, the survival of KC group was greater than that of SC group. We hypothesize that the loss of the ectopically transplanted human hepatocytes may be due in part to the absence of essential growth signals from human hepatocyte growth factor (HGF). Since HGF has a very short half-life (in the order of minutes), we used a c-Met agonistic antibody that activates the HGF pathway. When c-Met antibody was administered, persistent survival of the transplanted human hepatocytes in both KC and SC groups were seen for at least 100 days. This effect may be due to the multifunctional effects known to occur through HGF/c-Met signal transduction that affects trophic, proliferative, and cytoprotective responses on human hepatocytes.[8] Histological examination at 24 weeks posttransplantation revealed that the majority of the hepatocytes maintained their characteristic morphology (ie, single cell, large and eosinophilic cytoplasm, single or binuclei). Among those factors we determined the role of hepatocellular proliferation in grafted

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Table 1. BrdU Labeling Index in the Ectopically Transplanted Human Hepatocytes in Mice

Transplant Site	cMet Injection	BrdU L.I.
Kidney capsule	−(n = 3)	5.1 ± 1.0*
	+(n = 3)	32.6 ± 2.0*
Subcutaneous	−(n = 2)	2.6 (2.2, 2.9)
	+(n = 2)	23.0 (21.9, 24.0)

*$P < .01$ by Student's *t* test.

hepatocyte stabilization. We delivered BrdU, a marker for the proliferating cells after transplantation, and found that 20 to 35% of the hepatocytes were labeled over a period of 2 weeks as compared with 2% to 6% in the control group (Table 1). Because the level of human serum reporter molecules (eg, hAAT) remained the same throughout the study, our results suggested that cellular turnover is indeed one of the key factors for stabilizing hepatocellular function.

DISCUSSION

This study has shown that human hepatocytes can be engrafted ectopically and can function long term with the stimulation of c-Met. Furthermore, we have confirmed that c-Met signal transduction provided a proliferative stimulus for the transplanted hepatocytes in vivo. Both of these findings advance the future application of clinical hepatocyte transplantation regardless of the selection of the transplantation site. Ectopic sites offer the potential for achieving a larger number of viable hepatocytes necessary to complement hepatic function.

Finally, the study of the human hepatocyte-specific functions, have been greatly hampered by differences between their in vivo and in vitro properties. The established mouse system with human hepatocytes described here would be a great alternative, allowing us to study human hepatocyte-specific functions long term in vivo.[6]

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