Extracellular Matrix Component Cotransplantation Prolongs Survival of Heterotopically Transplanted Human Hepatocytes in Mice

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Engineering liver tissue using hepatocyte transplantation at extrahepatic (heterotopic) sites is important because a functioning extrahepatic liver would reduce the need for organ replacement to the number of patients with liver diseases.\textsuperscript{1,2} However, many attempts to achieve long-term maintenance of hepatocytes engrafted at the heterotopic sites have been unsuccessful.\textsuperscript{2} One of the major reasons for this poor survival could be the lack of the proper extracellular matrix proteins necessary for hepatocyte attachment and differentiation. In the culture experiment reported by Bissell et al.,\textsuperscript{3} hepatocytes were maintained in their differentiated status long-term when cells were cultured on extracellular matrix-gel (Matrigel)-coated dishes. The present study was designed to determine the effect of providing extracellular matrix components to promote the survival of human hepatocytes transplanted into the subcutaneous space and under the kidney capsule in mice.

MATERIALS AND METHODS

Female NOD/SCID recipient mice were purchased from Jackson (Bar Harbor, Me, USA) and housed under the institutional guidelines set forth by the Stanford University Animal Care Committee. Human hepatocytes were isolated from surgical liver specimens of noncancer cases using a two-step collagenase perfusion method as described previously.\textsuperscript{4} Hepatocytes were purified by three rounds of low-speed centrifugation at 50 g. Right before cell transplantation, the hepatocytes (1 × 10\textsuperscript{7} cells per milliliter) were resuspended in cold Williams E Medium (Invitrogen Corp, Carlsbad, Calif, USA) without serum or in an equal volume of Williams E Medium and cold extracellular matrix gel, Matrigel (BD Biosciences, Bedford, Mass, USA). Hepatocytes (4 × 10\textsuperscript{6} in 0.4 mL suspension were transplanted bilaterally under the kidney capsule spaces, or 6 × 10\textsuperscript{6} hepatocytes in 0.6 mL were transplanted into the subcutaneous space between the scapulae. The viability and survival of the transplanted hepatocytes in vivo were assessed by periodic mouse serum measurement of a human hepatocyte-specific serum marker, human alpha-1-antitrypsin (hAAT), as described previously.\textsuperscript{4} Mice were sacrificed at day 60 in the subcutaneous experiment and at day 120 in the kidney capsule experiment. Grafts were excised and processed for histological examination.

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For statistical analyses between the groups, the unpaired Student t test was employed. \( P < .05 \) was considered statistically significant.

RESULTS
Survival of human hepatocytes transplanted into the subcutaneous space sharply dropped to less than 1% over a period of 50 days. When the extracellular matrix components were provided to the hepatocytes by cotransplanting cells with Matrigel, significantly higher survival was obtained at time points measured from day 7 until the end of the experiment (Fig 1A). Hepatocytes transplanted under the kidney capsule showed better survival compared with the subcutaneous space. In addition, cotransplantation of hepatocytes with Matrigel under the kidney capsule allowed significantly higher survival compared with the non-Matrigel group; more than 10% of the hepatocytes survived for over 100 days (Fig 1B). Histologically, cells that retained hepatocyte-specific morphology were identified in the Matrigel groups with both subcutaneous and kidney capsule transplantation, while hepatocytes were hardly visible in the non-Matrigel groups.

DISCUSSION
The present study demonstrated that human hepatocytes transplanted into the subcutaneous space or under the kidney capsule survived significantly longer when extracellular matrix components were provided to the grafts. It has been reported that Matrigel is effective for cell attachment and differentiation of hepatocytes in vitro. Because the effects of Matrigel on hepatocyte survival were obtained from the early time point (day 7 after transplantation), it may be reasonable to speculate that Matrigel supported hepatocyte attachment at the extrahepatic sites in vivo. Furthermore, maintenance of a differentiated status and morphology as a mature hepatocyte was achieved by a long-term effect of Matrigel cotransplantation. The major extracellular matrix components of Matrigel are laminin, followed by collagen type IV, heparan sulfate proteoglycans, and entactin. The present study did not address which component is the major contributor to the prolonged hepatocyte survival. Further studies are required to identify the major contributory matrix components and to develop clinically applicable, artificial extracellular matrix mixtures specifically effective for hepatocyte transplantation and liver tissue engineering.

In consistent with our previous report and other reports, it is difficult to achieve persistent survival of hepatocytes transplanted into the subcutaneous space. The subcutaneous space is an important site for cell transplantation, because it has a large capacity. Unfortunately, this site is generally poor in a local vascular network; this poor vascularity could be the likely possibility for the low cell survival. For cells to survive long term in the subcutaneous space, more intensive studies are required to establish a local vascular network prior to cell transplantation.

REFERENCES