ELSEVIER

Heterotopically Transplanted Hepatocyte Survival Depends on Extracellular Matrix Components

K. Ohashi, M.A. Kay, H. Kuge, T. Yokoyama, H. Kanehiro, M. Hisanaga, S. Ko, M. Nagao, M. Sho, Y. Nakajima

ABSTRACT

The novel approach of tissue engineering to treat many forms of liver diseases using hepatocytes requires sufficient numbers and sustained survival of the transplanted cells. It has been shown that providing extracellular matrix components extracted from Engelbreth-Holm-Swarm cells (EHS-ECMs) to heterotopically transplanted hepatocytes allows significantly greater hepatocyte survival. We investigated the survival and morphology of hepatocytes and EHS-ECMs transplanted under the kidney capsule compared with hepatocytes with growth factor-reduced EHS-ECMs in mice. Both the EHS-ECMs and growth factor-reduced EHS-ECMs showed a large number of surviving hepatocytes under the kidney capsule without any intergroup differences. Histologically, transplanted hepatocytes in both groups retained their characteristic morphologies and formed small liver tissues. These data indicate that extracellular matrix components are the predominant factor in EHS-ECMs required to maintain hepatocytes at heterotopic sites.

LIVER TISSUE ENGINEERING using hepatocyte transplantation represents a new generation of cell-based therapy for several forms of hepatic diseases. However, a major drawback in establishing this new modality is the inefficient survival of the transplanted hepatocytes at heterotopic sites.[1] Loss of transplanted hepatocytes occurring within several days of grafting is likely due to the lack of appropriate extracellular matrix components (ECMs) needed for the cells to attach and accommodate.[1,2] We recently reported that the inefficient hepatocyte survival may be overcome by providing ECMs extracted from murine Engelbreth-Holm-Swarm tumor cell lines (EHS-ECMs).[3–6] The EHS-ECMs are rich in ECM components, predominantly laminin and type IV collagen, but also contain a small amount of growth factors, such as epidermal growth factor. This study was performed to determine the effects of EHS-ECMs on hepatocyte survival and whether it was solely due to ECMs or to a combination of ECMs and growth factors in EHS-ECMs.

MATERIALS AND METHODS

Using two-step collagenase perfusion methods, hepatocytes were isolated from transgenic mice that express human α1-antitrypsin as a marker protein under the hepatocyte-specific promoter (kindly provided by Dr Bumgardner, Ohio State University).[7] Hepatocyte purification was performed by three rounds of low-speed centrifugation at 50 *g*. Three million hepatocytes were resuspended in 140 μL of cold William's Medium E (group 1); 70 μL of medium and 70 μL of EHS-ECMs (Matrigel Matrix, BD Biosciences, Bedford, Mass, USA; group 2); and 70 μL of medium and 70 μL of growth factor–reduced EHS-ECMs (growth factor–reduced Matrigel Matrix, BD Biosciences; group 3). Cells were then transplanted by dividing the dose into the bilateral kidney capsule spaces of wild-type isogenic mice. Since the marker protein of the transplanted hepatocytes, hAAT, demonstrates a short half-life (less than 2 hours) in the mouse[7] and is produced only from the hepatocytes, the viability and survival of the transplanted hepatocytes in vivo can reasonably be assessed by periodic mouse serum measurement of the hAAT reporter protein, as described previously.[3–7] Mice were sacrificed at day 140; the grafts were excised and processed for histological examination.

From the Department of Surgery, Nara Medical University, Nara, Japan.

This work was supported by NIH U19-AI40034 (M.A.K.), grant-in-aid from the Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (Y.N. and K.O.), and Japan Society for the Promotion of Science Fellowship (K.O.).

Address reprint requests to Kazuo Ohashi, MD, PhD, Department of Surgery, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634-8522, Japan. E-mail: kohashi@naramed-u.ac.jp

0041-1345/05/$–see front matter
doi:10.1016/j.transproceed.2005.11.049

RESULTS

The survival of transplanted hepatocytes without ECMs (group 1), determined by the serum hAAT levels, sharply declined to 40% ± 10.5% of the day 2 levels at day 7 and gradually decreased afterward (Fig 1). In our previous reports, significantly higher hepatocyte survival was achieved when human or mouse hepatocytes were cotransplanted with EHS-ECMs at heterotopic sites as compared to hepatocytes without ECMs.[1,4,5] In line with these data, significantly higher and sustained survivals were achieved in group 2 (hepatocytes with EHS-ECMs) compared with the survival of hepatocytes alone (group 1). When hepatocytes were transplanted with growth factor–reduced EHS-ECMs (group 3), sustained survival was also achieved with no difference in survival compared to group 2. Histological examination of the specimens obtained at 140 days revealed that hepatocytes retained hepatocyte-specific morphologies, forming small liver tissues with liverlike and capillarized architecture in both groups 2 and 3.

DISCUSSION

One of the major paradigms of liver tissue engineering in hepatocyte transplantation seeks to achieve sufficient cell engraftment and survival.[1,3] In the liver, hepatocytes are surrounded by ECMs that are important for functional and structural maintenance through cell–cell and cell–ECM interactions.[8] However, once hepatocytes are isolated from their natural environment, they detach from the ECMs and undergo apoptosis through detachment-induced cell death that occurs within a short period after isolation.[2] Although the detailed molecular mechanisms involved in this type of cell death have not fully been understood, the primary factors are the lack of active survival signals, caused by the functional loss of β1-integrin–dependent signal pathways and loss of cell attachment. In this context, we provided ECMs to the transplanted hepatocytes at a heterotopic site as a possible way to avoid hepatocyte apoptosis. The present study clearly demonstrated that providing EHS-ECMs in the transplant setting contributed to increased hepatocyte engraftment as well as to their stable survival, leading to engineered liver tissue.

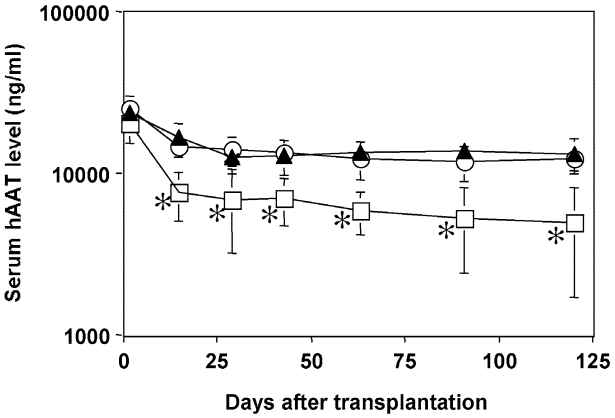

Fig 1. Survival of hepatocytes transplanted under the kidney capsule in mice. Open square (group 1): mice received hepatocytes resuspended with medium only; open circle (group 2): mice received hepatocytes resuspended with EHS-ECMs; closed triangle (group 3): mice received hepatocytes resuspended with growth factor-reduced EHS-ECMs. *$P < .05$ between group 1 versus the other two groups.

Laminin and type IV collagen are the major components of EHS-ECMs. However, they also contain small amounts of EGF and IGF-1. The present data showed no remarkable differences in the survival of groups 2 and 3, suggesting that ECMs are the predominant contributory factor for the efficient engraftment and persistence of hepatocytes at a heterotopic site. Maintenance of function and the differentiation status of hepatocytes have also been reported in culture studies by providing laminin and type IV collagen.[9,10] Further studies are required to elucidate the molecular mechanisms of apoptosis in isolated hepatocytes with respect to cell–cell extracellular matrix interactions. However, our approach achieved successful hepatocyte engraftment that may further advances in liver tissue engineering.

REFERENCES

1. Ohashi K, Park F, Kay MA: Hepatocyte transplantation: clinical and experimental application. J Mol Med 79:617, 2001
2. Zvibel I, Smets F, Soriano H: Anoikis: roadback to cell transplantation. Cell Transplantation 11:621, 2002
3. Ohashi K, Marion PL, Nakai H, et al. Sustained survival of human hepatocytes in mice: a model for in vivo infection with human hepatitis B and hepatitis delta viruses. Nat Med 6:327, 2000
4. Ohashi K, Kay MA, Yokoyama T, et al: Stability and repeat regeneration potential of the engineered liver tissues under the kidney capsule in mice. Cell Transplantation in press, 2005
5. Ohashi K, Kay MA: Extracellular matrix co-transplantation prolongs survival of heterotopically transplanted human hepatocytes in mice. Transplant Proc 36:2469, 2004
6. Ohashi K, Waugh JM, Dake MD, et al: Liver tissue engineering at extra-hepatic sites in mice as a potential new therapy for genetic liver diseases. Hepatology 41:132, 2005
7. Bumgardner GL, Heininger M, Li J, et al: A functional model of hepatocyte transplantation for in vivo immunologic studies. Transplantation 65:53, 1998
8. Greenwel P, Rojkind M: The extracellular matrix of the liver. In Arias IM, Boyer JL, Chisari FV, et al (eds): The Liver; Biology and Pathobiology. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001
9. Bissel DM, Arenson DM, Maher JJ, et al: Support of cultured hepatocytes by a laminin-rich gel. Evidence for a functionally significant subendothelial matrix in normal rat liver. J Clin Invest 79:801, 1987
10. Pinkse GGM, Voorhoeve MP, Noteborn M, et al: Hepatocyte survival depends on β1-integrin-mediated attachment of hepatocytes to hepatic extracellular matrix. Liver International 24:218, 2004