TECHNICAL REPORTS

nature medicine

Isolated small intestinal segments support auxiliary livers with maintenance of hepatic functions

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We determine here the functional integrity of auxiliary livers in containers fashioned from the small intestine. Liver microfragments from dipeptidyl peptidase 4 (DPP4)-deficient rats were transplanted into syngeneic normal animals with isolated intestinal segments characterized by mucosal denudation but intact vascular supply. Transplanted liver fragments were restored to confluent tissue with normal hepatic architecture and development of DPP4-positive vessels, indicating angiogenesis and revascularization. Auxiliary liver units expressed multiple hepatotrophic and angiogenic genes, and transplanted tissues remained intact for up to the 6-week duration of the studies with neither ischemic injury nor significant hepatocellular proliferation. Hepatic metabolic, transport and synthetic functions were preserved in auxiliary livers, including uptake and biliary excretion of ^{99m}Tc-mebrofenin in syngeneic recipients of liver from F344 rats, as well as secretion of albumin in allografted Nagase analbuminemic rats. This ability to produce functionally competent auxiliary livers in vascularized intestinal segments offers therapeutic potential for liver disease and genetic deficiency.

The disparity between the supply of and the demand for donor livers requires alternatives to orthotopic liver transplantation (OLT). In acute liver failure, in which the native liver may recover, and in deficiency states characterized by extrahepatic organ damage (for example, Crigler-Najjar syndrome and familial hypercholesterolemia[1]), auxiliary liver transplantation is more attractive than OLT[2–7]. However, livers in heterotopic locations atrophy without portal blood[8,9], whereas portal blood supply to transplanted liver in auxiliary partial OLT improves graft survival[2–7]. Simpler ways to produce auxiliary livers would be useful in clinical medicine. Transplantation of liver fragments subcutaneously or under the liver capsule was unsuccessful[10], although transplantation of liver cells into the spleen or peritoneal cavity has been more successful[10,11].

We recently established that suitable containers for heterotopic livers can be fashioned from vascularized segments of the small intestine[12]. In this system, a segment of the intestine with satisfactory vascular pedicle is isolated. After dividing the intestine at two places to isolate the segment, intestinal continuity is restored by anastomosis of the divided ends. Next, the mucosa of the intestinal segment is removed, one end of the segment is closed and the container is filled with liver microfragments. Here, we provide analysis of this intestinal heterotopic liver to establish mechanisms concerning reorganization and function of the transplanted liver.

RESULTS

Engraftment and fate of the transplanted liver

We used inbred syngeneic rats in the F344 background with analysis of three to five rats at each interval. Typically, a 2-cm long intestinal segment accommodated 0.3–0.5 g of liver, which represented ~5% of the native liver mass. Denudation of the intestinal mucosa was obvious on histological analysis of biocontainers. Liver fragments were initially separated from the intestinal wall by serosanguineous exudates (**Fig. 1**). Subsequently, transplanted tissue was reorganized and, after 10–12 d or 4–6 weeks, hepatic parenchyma was indistinguishable from normal liver lobules. Transplanted tissues contained healthy hepatocytes without steatosis or bile accumulation, although at later times portal areas showed mild to moderate fibrosis. Scanning electron microscopy showed that transplanted tissues were organized normally with sinusoids separating parenchymal cells. Moreover, γ-glutamyltranspeptidase (GGT) staining of transplanted liver showed reactivity in biliary cells but not in hepatocytes, whereas liver after vaso-occlusive ischemic injury showed extensive GGT expression. These findings indicated adequate perfusion of transplanted tissue. Moreover, histochemical methods verified the presence of albumin and glycogen in the transplanted liver, similar to normal liver.

To demonstrate remodeling in transplanted tissue requiring hepatocellular proliferation, we used the antibody Ki-67, which binds a non-histone nuclear protein expressed in all cell-cycle phases, except G0/early G1 (**Fig. 2**)[13]. Ki-67 staining was observed in only 0.9 ± 0.1%

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Published online 30 May 2004; doi:10.1038/nm1057

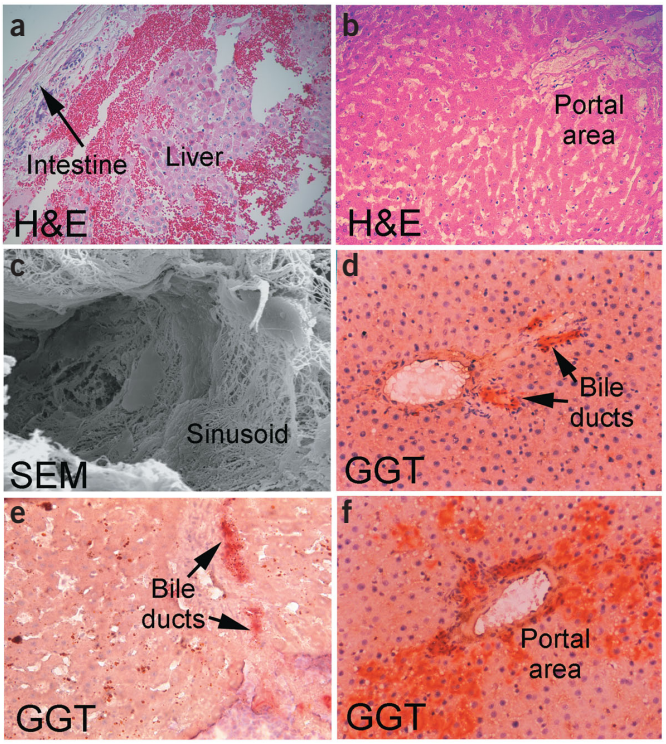

Figure 1 Histological analysis of auxiliary liver. Hemorrhagic exudates separated the intestinal submucosa from liver after 2 h (a), whereas transplanted liver was healthy after 12 d (b) and scanning electron microscopy (SEM) showed intact morphological organization at that time (c). GGT expression was restricted to bile ducts in normal liver (d) and transplanted liver 12 d after transplantation (e), but was expressed extensively in liver subjected to ischemia (f). Original magnification: a, ×100; c, ×2500; b,d–f, ×400. H&E, hematoxylin and eosin.

of cells in normal F344 rat liver, compared with 28.0 ± 1.1% of cells in rats 30 h after two-thirds partial hepatectomy ($P < 0.001$, t-test). In auxiliary livers, 4.1 ± 0.9% of hepatocytes stained with Ki-67 1 d after transplantation ($P < 0.001$, t-test), with no Ki-67 staining in liver 12 d or 6 weeks after transplantation. These findings indicated an absence of significant regenerative activity from auxiliary liver.

Revascularization of transplanted tissue

To demonstrate tissue revascularization, we transplanted liver from syngeneic rats deficient in DPP4 activity[14] into normal F344 rats. DPP4 is expressed ubiquitously in tissues, including endothelial cells, which provided a way to define whether transplanted tissue was revascularized from the host. The auxiliary liver showed DPP4 staining of intestinal mucosal and submucosal capillary networks disrupted during mucosal denudation (**Fig. 3a**). However, within 2–3 d after transplantation, DPP4-positive capillaries were observed at the periphery of liver tissue in the vicinity of intestinal walls. At later times, occasional DPP4-positive blood vessels were observed even in the transplanted liver parenchyma (**Fig. 3b**). Immunostaining for desmin and α-smooth muscle actin (SMA) showed mature blood vessels in the intestinal wall and transplanted liver throughout the study period (**Figs. 3c** and **d**, respectively). We analyzed corrosion casts of transplanted tissues following antegrade filling of vessels via the abdominal aorta to further demonstrate development of blood vessels (**Fig. 3e**). Immediately after deposition of liver fragments in intestinal containers, blood vessel ends showed a truncated appearance. However, 12 d after establishment of the auxiliary liver, networks of arborizing capillaries originating from proximal arterioles were visible, in agreement with angiogenesis.

Regulation of gene expression in tissues

To determine whether angiogenic and hepatotrophic genes regulated transplanted tissue survival, we carried out reverse transcription–polymerase chain reactions (RT-PCR; **Fig. 3f**). Tissue samples were taken from rats 5 d and 12 d after liver transplantation. The intestinal and hepatic portions of the auxiliary livers were collected separately by careful dissection. Expression of genes encoding multiple hepatotrophic growth factors was retained in the auxiliary liver, including those encoding hepatocyte growth factor (HGF), transforming growth factor (TGF)-α and fibroblast growth factor (FGF)-1, along with the corresponding receptors, except for FGF-1 receptor. Also, genes encoding TGF-β1 and TGF-β2 were expressed in auxiliary liver and its intestinal wall. Among angiogenesis regulatory genes, those encoding vascular endothelial growth factor (VEGF), angiopoietin (ANG)-1 and ANG-2, and the corresponding receptors, VEGFR-1, VEGFR-2 and TIE-2, were expressed in the auxiliary liver. VEGF, VEGFR-2 and TIE-2 were expressed to a lesser extent in the native liver and intestine. These findings were in agreement with angiogenesis, hepatotrophic activity and the possibility of appropriate regulatory interactions in the auxiliary liver.

Analysis of function in auxiliary liver

To ascertain the function of transplanted liver, we first analyzed handling of the organic anion mebrofenin, which is rapidly incorporated in the liver and transported into bile[15]. We expected secretion of radiolabeled mebrofenin incorporated in the auxiliary liver into the blood

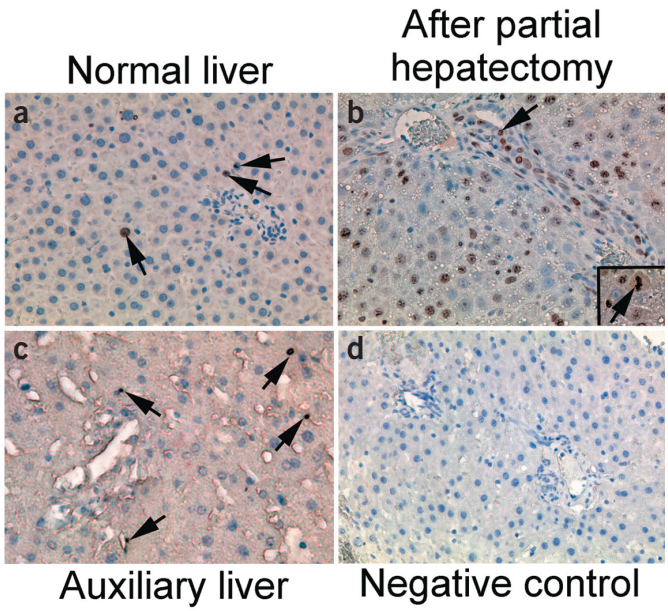

Figure 2 Ki-67 immunostaining of liver. Ki-67 staining was infrequent in the normal liver (arrows, **a**), whereas 30 h after partial hepatectomy, hepatocytes and nonparenchymal cells (arrow, **b**) showed extensive staining (inset, hepatic mitosis). Ki-67–stained hepatocytes were seen occasionally in the auxiliary liver after 1 d (arrows). Original magnification: **a**–**c**, ×400; **d**, ×200. Toluidine blue was used for counterstaining.

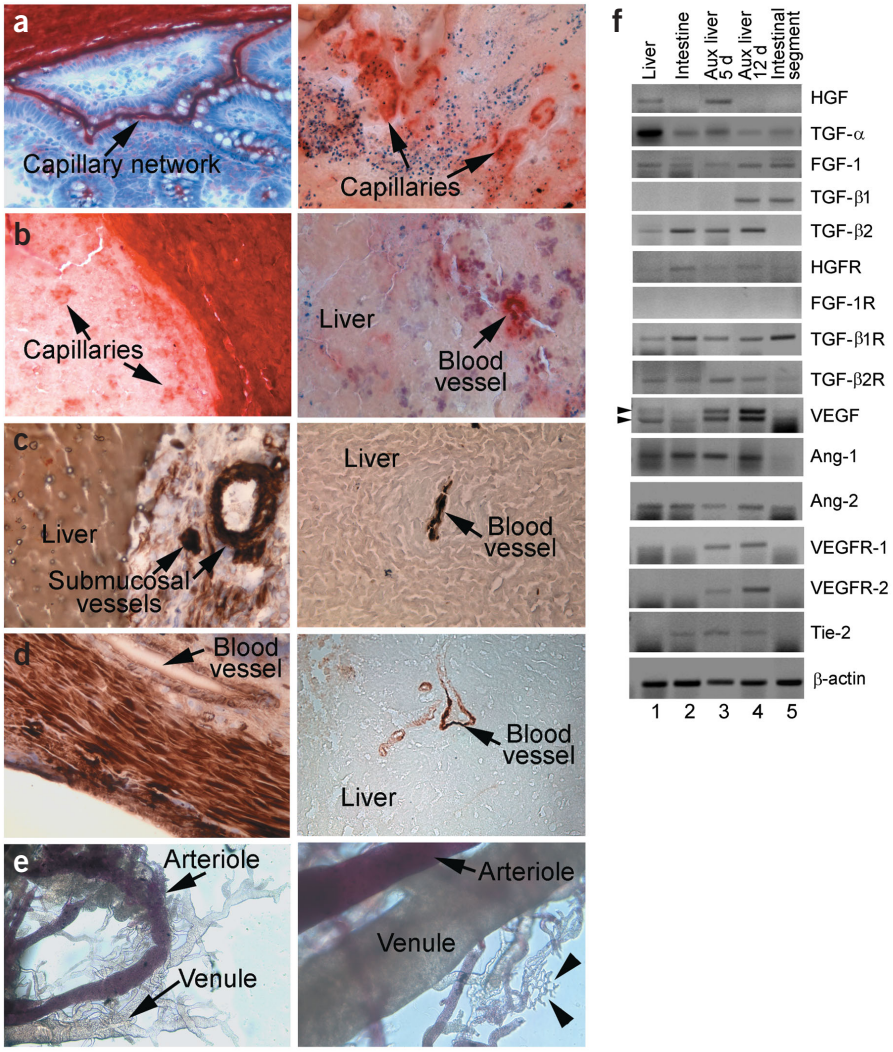

Figure 3 Revascularization of transplanted tissue after 12 d. (**a**) Native DPP4-positive capillaries in normal intestinal mucosa (left) and DPP4-deficient liver adjacent to intestinal wall (right). (**b**) DPP4-positive vessels next to the intestinal wall (left) and in the central part of transplanted DPP4-deficient liver (right). Also shown is desmin (**c**) and α-SMA (**d**) staining of vessels in intestinal wall (left) and auxiliary liver (right). (**e**) Corrosion casts immediately after liver transplantation showed vascular truncation (left), whereas capillary networks were formed after 12 d (arrowheads, right). (**f**) RT-PCR for hepatotrophic and angiogenic genes. VEGF165 and VEGF121 transcripts were amplified (arrowheads). Original magnification: **a**–**d**, ×400; **e**, ×100.

followed by its excretion by the native liver into bile. Mebrofenin was incorporated in the auxiliary liver, although the uptake was delayed relative to the native liver ($P < 0.01$, *t*-test; **Fig. 4**). Nonetheless, the kinetics of mebrofenin excretion were similar to those of the native liver, such that most of the mebrofenin was cleared within 30 min, similar to the rate of the normal liver[15].

We further examined secretion of hepatic proteins from the auxiliary liver into the circulation. For this purpose, we used Nagase analbuminemic rats (NAR) because they show extremely low albumin levels and constitute an authentic model of a monogenic disorder. When allogeneic normal rat liver was transplanted in NAR under cyclosporine[16], auxiliary livers retained albumin expression (**Fig. 5**). Moreover, serum albumin was detectable in the rats for the 6-week duration of the study. Densitometric analysis of western blots showed serum albumin levels in treated NAR of ~1% of normal. These data demonstrated the functional integrity of a significant fraction of transplanted liver over prolonged periods.

DISCUSSION

We have established here that vascularized small intestinal segments denuded of the mucosa provide a unique microenvironment for supporting transplanted liver. This is distinct from transplantation of specific liver cell populations, as well as creation of bioartificial liver devices using cells, because hepatic morphology and relationships between parenchymal and nonparenchymal liver cells were maintained[10,11]. Moreover, transplanted liver retained metabolic function (glycogen synthesis), secretory function (albumin synthesis) and hepatobiliary excretory function (mebrofenin transport), which are all appropriate elements for successful applications of auxiliary livers.

The evidence for revascularization of transplanted tissue in intestinal segments was noteworthy, especially without portal blood, which contains putative hepatotrophic factors[8,9], including pancreatic hormones and substrates for intermediary metabolism. The small intestinal wall has a predilection for endothelialization as shown in studies using jejunal submucosa to generate vascular grafts[17]. Our studies provide evidence for angiogenesis in transplanted liver. DPP4-deficient F344 rats were effective for this demonstration, as were corrosion casts of vessels. The additional use of the DPP4-deficient rat, which has been valuable for studying transplanted cell biology[14,16], for demonstrating angiogenesis in the auxiliary liver should permit further analysis of related mechanisms. Major angiogenic genes were expressed in transplanted liver and, to a limited extent, in the intestinal wall. Among these, VEGF has been well studied for its role in angiogenesis and its potential for therapeutic vasculogenesis[18]. The major forms of VEGF expressed in transplanted liver exhibit potent angiogenic properties in animals. The roles of VEGF in angiogenesis include vascular permeabilization and dilatation, loosening of endothelial cell junctions, recruitment of facilitatory cells and regulation of endothelial cell responses[19]. The signaling system regulating vessel growth, stabilization and maintenance is heavily dependent on further interactions between ANG-1 and ANG-2, which interact with the endothelial receptor tyrosine kinase TIE-2 to either promote or impede angiogenesis, depending on the context in specific organs[20]. Therefore, expression of ANG-1, ANG-2 and TIE-2 in the auxiliary liver should be consistent with roles for these molecules in mediating angiogenesis.

Cell-cell interactions, as well as extracellular matrix components, regulate hepatocyte survival in heterotopic locations[21]. Extracellular matrix components and additional signals may have originated from the small intestinal component of the auxiliary liver during

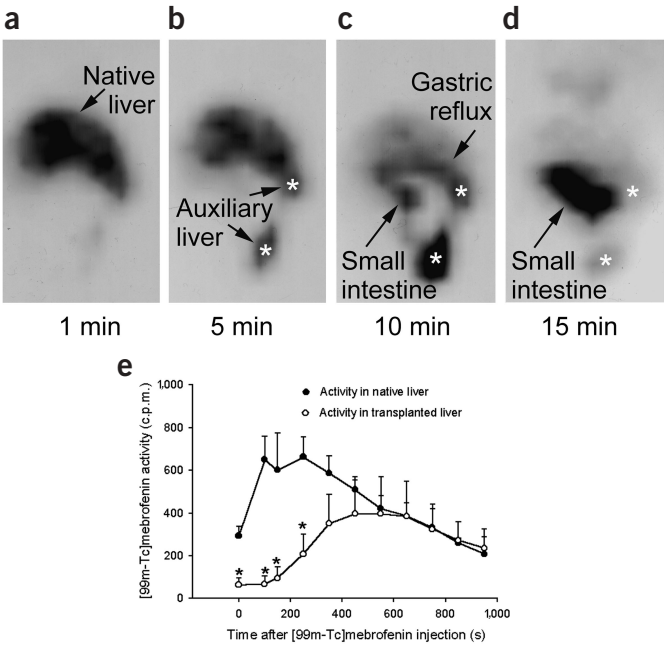

Figure 4 ^{99m}Tc-mebrofenin handling in transplanted liver. Imaging of rats 30 d after transplantation showing delayed mebrofenin uptake in the auxiliary liver (**a**,**b**), although subsequently mebrofenin was promptly excreted into the gut from both auxiliary and native livers (**c**,**d**). (**e**) Kinetics of mebrofenin handling in five rats 30 d after liver transplantation. $^*P < 0.05$, *t*-test. Asterisks in panels **b**–**d** indicate the position of the auxiliary liver.

angiogenesis. Also, the shared embryonic foregut origin of liver and intestine suggests the potential for cell-cell interactions. For instance, intestinal subepithelial myofibroblasts participate in epithelial-mesenchymal interactions, in part by secreting HGF and TGF-β[22]. We observed α-SMA–reactive myofibroblasts in the intestinal wall of auxiliary livers with TGF-α, FGF-1 and TGF-β expression. LeCouter *et al.*[23] demonstrated VEGF-induced communication by means of VEGFR-1 between endothelial cells and hepatocytes involving the soluble signals HGF and interleukin-6. This was associated with hepatocyte proliferation without endothelial cell proliferation. On the other hand, VEGFR-2 activation increased endothelial cell proliferation without hepatocyte proliferation. Such interactions likely occurred in the auxiliary liver, as both VEGF receptors were expressed. We consider it likely that survival of liver in intestinal segments resulted from superior revascularization and angiogenic signaling. The hepatoprotective properties of VEGFR-1 against carbon tetrachloride-induced toxicity support this possibility[23]. Additional hepatotrophic genes and their receptors in auxiliary livers might promote survival of transplanted livers. In particular, HGF has potent roles as a hepatic morphogen and mitogen and in angiogenesis[20]. Similarly, TGF-α promotes hepatocyte proliferation. However, limited DNA synthesis in the transplanted liver was in agreement with coordinated regulation of liver growth, also suggested by TGF-β expression, which inhibits hepatocellular proliferation.

The incorporation of mebrofenin in the auxiliary liver followed by its release verified the integrity of both arterial and venous limbs of the circulation and provided evidence for biliary excretory function in transplanted tissue. However, the final bile clearance was dependent on the native liver. This was similar to biliary excretion from transplanted hepatocytes in ectopic sites, for example, spleen, or even in the liver itself, where reconstitution of functionally intact hybrid bile canaliculi permits bile excretion[14]. In principle, specific tracers, including ^{99m}Tc-mebrofenin and tagged bile salt analogs, may help establish the kinetics of bile production in the auxiliary liver, for example, by imaging and by biochemical methods for analyzing the venous effluent.

The extent of function in auxiliary liver was appropriate for the liver mass transplanted. In response to transplantation of ~5% liver mass, we found that auxiliary livers reconstituted ~1% of serum albumin levels in NAR. Although allografted NAR were immunosuppressed, use of cyclosporine alone was neither optimal nor monitored by blood levels of the drug, in view of practical difficulties. Therefore, our results in NAR likely represent minimal efficacies. These results should improve with superior vascularization and regeneration in transplanted liver, as well as by transplanting larger masses of liver (for example, into multiple intestinal segments). We examined the feasibility of the latter procedure in rats, although analysis of transplanted liver in multiple segments requires more work.

The auxiliary liver described here may be helpful in the areas of liver regeneration, toxicology and tissue engineering. Potential sources of

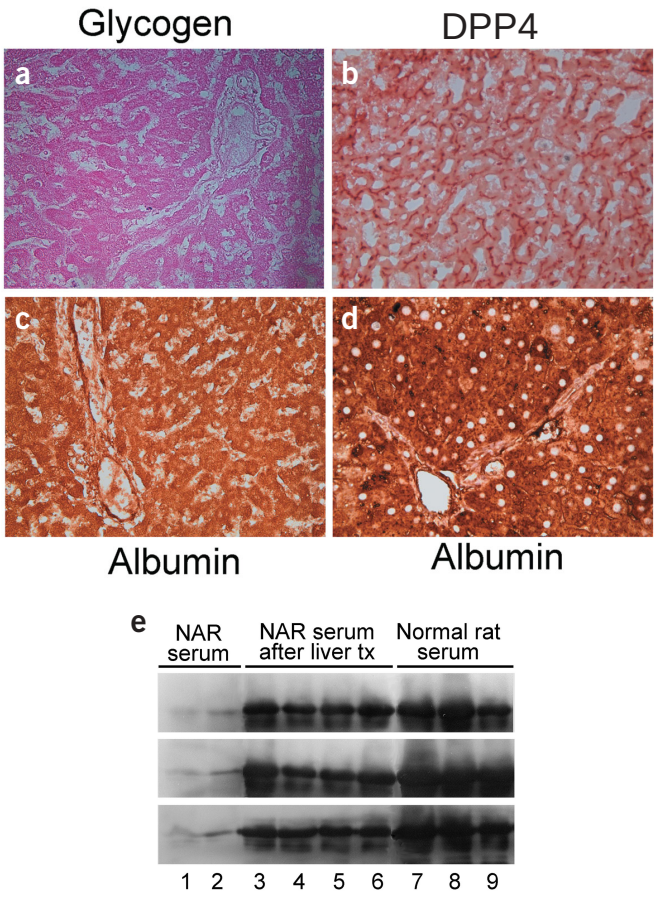

Figure 5 Function of auxiliary liver in NAR. Liver 6 d after transplantation showed the presence of glycogen and albumin in hepatocytes and DPP4 activity in bile canaliculi (**a**–**c**, transplanted liver; **d**, normal liver). (**e**) Western blots of serial serum samples from three of six NAR studied. Lanes 1 and 2, pretransplantation samples showing virtually no albumin; lanes 3–6, sera 1, 2, 3 and 6 weeks after liver transplantation; lanes 7–9, donor rat serum with 1:200, 1:400 and 1:800 dilutions, respectively.

donor liver for this purpose include fetal human and xenogeneic tissues to avoid depletion of human livers suitable for OLT. Of course, analysis of this auxiliary liver in additional disorders, including liver failure or metabolic diseases, will be appropriate.

METHODS

Animals. We obtained F344 rats from the National Cancer Institute, Bethesda, Maryland. DPP4-deficient rats, NAR and inbred Long-Evans Agouti rats were from the Special Animal Core of the Marion Bessin Liver Research Center. The rats were 6 to 10 weeks old and weighed 150–300 g. Animals were housed in 12-h day-and-night cycles with pelleted diet and water *ad libitum*. Animal Care and Use Committees at Albert Einstein College of Medicine and Long Island Jewish Hospital approved animal procedures. Preparation of liver microfragments and surgery was as described[12], except donor livers were from either DPP4-deficient rats or Long-Evans Agouti rats, the latter for NAR studies. NAR were immunosuppressed with 10 mg/kg cyclosporine by intramuscular injections twice weekly[16]. Groups of 3–5 rats were killed at various intervals. To induce ischemic hepatic injury for demonstrating GGT activation, primary rat hepatocytes were transplanted intrasplenically[16]. We carried out two-thirds partial hepatectomy in adult F344 rats using the standard Higgins and Anderson technique.

Histological studies. Formalin-fixed tissues were paraffin-embedded and sectioned using standard methods. Tissues were frozen in methylbutane at –80 °C for cryosections. Histochemical staining was carried out for glycogen, DPP4 and GGT[16]. Tissues were immunostained with antibodies to rat albumin (1:500, United States Biochemicals), α-SMA (1:400, Sigma), desmin (1:50, Dako Scientific) and Ki-67 (1:100, Pharmingen), with detection using peroxidase-conjugated, rabbit-specific goat IgG for albumin (1:200, Sigma) and mouse-specific goat IgG for α-SMA, desmin and Ki-67 (1:150, Sigma) using DAB (K3465 kit, Dako). Morphometry for cell proliferation used analysis of nuclei in 10–12 contiguous fields under ×400 magnification from each tissue sample. For electron microscopy, tissues were fixed in glutaraldehyde in cacodylate buffer and freeze-fracture preparations were coated with gold.

Vascular casts. The abdominal aorta was cannulated, blood was flushed out with normal saline, and 3 ml 50% latex in water (NAIRIT L3, NAIRIT) was injected under 12 cm H_2O pressure. Tissue was digested in 80% HCl for 8–10 d at room temperature and casts were washed in water.

RNA extraction and RT-PCR analysis. Total cellular RNA was extracted with the Trizol reagent (Life Technologies). We used published primer sequences (HGF[24]; HGFR[25]; TGF-α[26]; FGF-1 and FGF-1R[27]; TGF-β[28]; VEGF, VEGFR-1 and VEGFR-2 (ref. 29); and Ang-1, Ang-2 and Tie-2 (ref. 30)). RNAs (1 μg) were reverse-transcribed and amplified in a total volume of 50 μl using 1× PCR buffer, 10 U reverse transcriptase, 100 U RNase inhibitor, 1.25 μg oligodT primer and dNTPs (Omniscript RT PCR kit). Reverse transcription products were amplified by 35 PCR cycles (Platinum PCR kit, Invitrogen). Products were resolved in agarose gels containing ethidium bromide.

^{99m}Tc-mebrofenin incorporation analysis. A commercial kit was used to mix 185–222 MBq ^{99m}Tc-sodium pertechnetate with mebrofenin (Choletec, Bracco Diagnostics). Each rat received a 100 μCi dose intravenously into a peripheral vein. Gamma imaging and image analysis were carried out as described[15].

Western-blot analysis. Sera were resolved by denaturing SDS-PAGE using 10% gels. Western blots were probed with antibody to rat albumin (1:10,000 dilution, United States Biochemicals) and developed with peroxidase-conjugated, rabbit-specific goat IgG (1:15,000, Sigma) using chemiluminiscence. We used the ImageJ 1.28u software program (NIH) for densitometry.

Statistical analysis. Data are expressed as means ± s.d., where appropriate, and were analyzed for statistical significance with Student's *t*-tests. P values < 0.05 were considered significant.

ACKNOWLEDGMENTS

We thank C. Zhang for technical assistance. This work was supported in part by NIH grants R01 DK46952 and P30-DK-41296 and by grant G-362 from the International Scientific Technology Center, Moscow, Russia.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Received 12 September 2003; accepted 26 April 2004
Published online at http://www.nature.com/naturemedicine/

1. Kayler, L.K. *et al.* Long-term survival after liver transplantation in children with metabolic disorders. *Pediatr. Transplant.* **6**, 295–300 (2002).
2. Azoulay, D. *et al.* Auxiliary partial orthotopic versus standard orthotopic whole liver transplantation for acute liver failure: a reappraisal from a single center by a case-control study. *Ann. Surg.* **234**, 723–731 (2001).
3. Kiuchi, T. *et al.* Auxiliary liver transplantation for urea-cycle enzyme deficiencies: lessons from three cases. *Transplant. Proc.* **31**, 528–529 (1999).
4. Rela, M. *et al.* Auxiliary partial orthotopic liver transplantation for Crigler–Najjar syndrome type I. *Ann. Surg.* **229**, 565–569 (1999).
5. Angelis, M., Pegelow, C.H., Khan, F.A., Verzaro, R. & Tzakis, A.G. En bloc heterotopic auxiliary liver and bilateral renal transplant in a patient with homozygous protein C deficiency. *J. Pediatr.* **138**, 120–122 (2001).
6. Haberal, M. *et al.* Successful heterotopic segmental liver transplantation from a live donor to a patient with Alagille syndrome. *J. Pediatr. Surg.* **36**, 667–671 (2001).
7. Durand, F. *et al.* Auxiliary liver transplantation for fulminant hepatitis B: Results from a series of six patients with special emphasis on regeneration and recurrence of hepatitis B. *Liver Transpl.* **8**, 701–707 (2002).
8. Kaibori, M. *et al.* Selective portal blood flow diversion in auxiliary partial orthotopic liver transplantation to induce regeneration of the graft. *Transplantation* **66**, 935–937 (1998).
9. de Jonge, J. *et al.* Directing portal flow is essential for graft survival in auxiliary partial heterotopic liver transplantation in the dog. *J. Pediatr. Surg.* **34**, 1265–1268 (1999).
10. Gupta, S. & Roy Chowdhury, J. Hepatocyte transplantation: Back to the future! *Hepatology* **15**, 156–162 (1992).
11. Sundback, C.A. & Vacanti, J.P. Alternatives to liver transplantation: from hepatocyte transplantation to tissue-engineered organs. *Gastroenterology* **118**, 438–442 (2000).
12. Berishvili, E. *et al.* Generation of heterotopic liver in an isolated and vascularized segment of the small intestine in rats. *Transplantation* **75**, 1827–1832 (2003).
13. Gaglio, P.J. *et al.* Liver regeneration investigated in a non-human primate model (*Macaca mulatta*). *J. Hepatol.* **37**, 625–632 (2002).
14. Gupta, S., Rajvanshi, P. & Lee, C.-D. Integration of transplanted hepatocytes in host liver plates demonstrated with dipeptidyl peptidase IV deficient rats. *Proc. Natl. Acad. Sci. USA* **92**, 5860–5864 (1995).
15. Malhi, H. *et al.* ^{99m}Tc-mebrofenin scintiscanning for evaluating liver disease in a rat model of Wilson's disease. *J. Nucl. Med.* **43**, 246–252 (2002).
16. Rajvanshi, P., Kerr, A., Bhargava, K.K., Burk, R.D. & 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* **23**, 482–496 (1996).
17. Robotin-Johnson, M.C., Swanson, P.E., Johnson, D.C., Schuessler, R.B. & Cox, J.L. An experimental model of small intestinal submucosa as a growing vascular graft. *J. Thorac. Cardiovasc. Surg.* **116**, 805–811 (1998).
18. Yla-Herttuala, S. & Alitalo, K. Gene transfer as a tool to induce therapeutic vascular growth. *Nat. Med.* **9**, 694–695 (2003).
19. Ferrara, N., Gerber, H.P. & LeCouter, J. The biology of VEGF and its receptors. *Nat. Med.* **9**, 669–676 (2003).
20. Cleaver, O. & Melton, D.A. Endothelial signaling during development. *Nat. Med.* **9**, 661–668 (2003).
21. Selden, C. *et al.* Histidinemia in mice: a metabolic defect treated using a novel approach to hepatocellular transplantation. *Hepatology* **21**, 1405–1412 (1995).
22. Powell, D.W. *et al.* Myofibroblasts. II. Intestinal subepithelial myofibroblasts. *Am. J. Physiol.* **277**, C183–C201 (1999).
23. LeCouter, J. *et al.* Angiogenesis-independent endothelial protection of liver: role of VEGFR-1. *Science* **299**, 890–893 (2003).
24. Suzuki, S. *et al.* Skeletal muscle injury induces hepatocyte growth factor expression in spleen. *Biochem. Biophys. Res. Commun.* **292**, 709–714 (2002).
25. Oh, S.H. *et al.* Hepatocyte growth factor induces differentiation of adult rat bone marrow cells into a hepatocyte lineage *in vitro*. *Biochem. Biophys. Res. Commun.* **279**, 500–504 (2000).
26. Scotte, M. *et al.* Cytokine gene expression in liver following minor or major hepatectomy in rat. *Cytokine* **9**, 859–867 (1997).
27. Kalyani, A.J., Mujtaba, T. & Rao, M.S. Expression of EGF receptor and FGF receptor isoforms during neuroepithelial stem cell differentiation. *J. Neurobiol.* **38**, 207–224 (1999).
28. Zhao, H., Patra, A., Tanaka, Y., Li, L.C. & Dahiya, R. Transforming growth factor-β(s) and their receptors in aging rat prostate. *Biochem. Biophys. Res. Commun.* **294**, 464–469 (2002).
29. Ishii, H., Oota, I., Arakawa, T. & Takuma, T. Differential gene expression of vascular endothelial growth factor isoforms and their receptors in the development of the rat masseter muscle. *Arch. Oral. Biol.* **47**, 505–510 (2002).
30. Haggstrom Rudolfsson, S., Johansson, A., Franck Lissbrant, I., Wikstrom, P. & Bergh, A. Localized expression of angiopoietin 1 and 2 may explain unique characteristics of the rat testicular microvasculature. *Biol. Reprod.* **69**, 1231–1237 (2003).