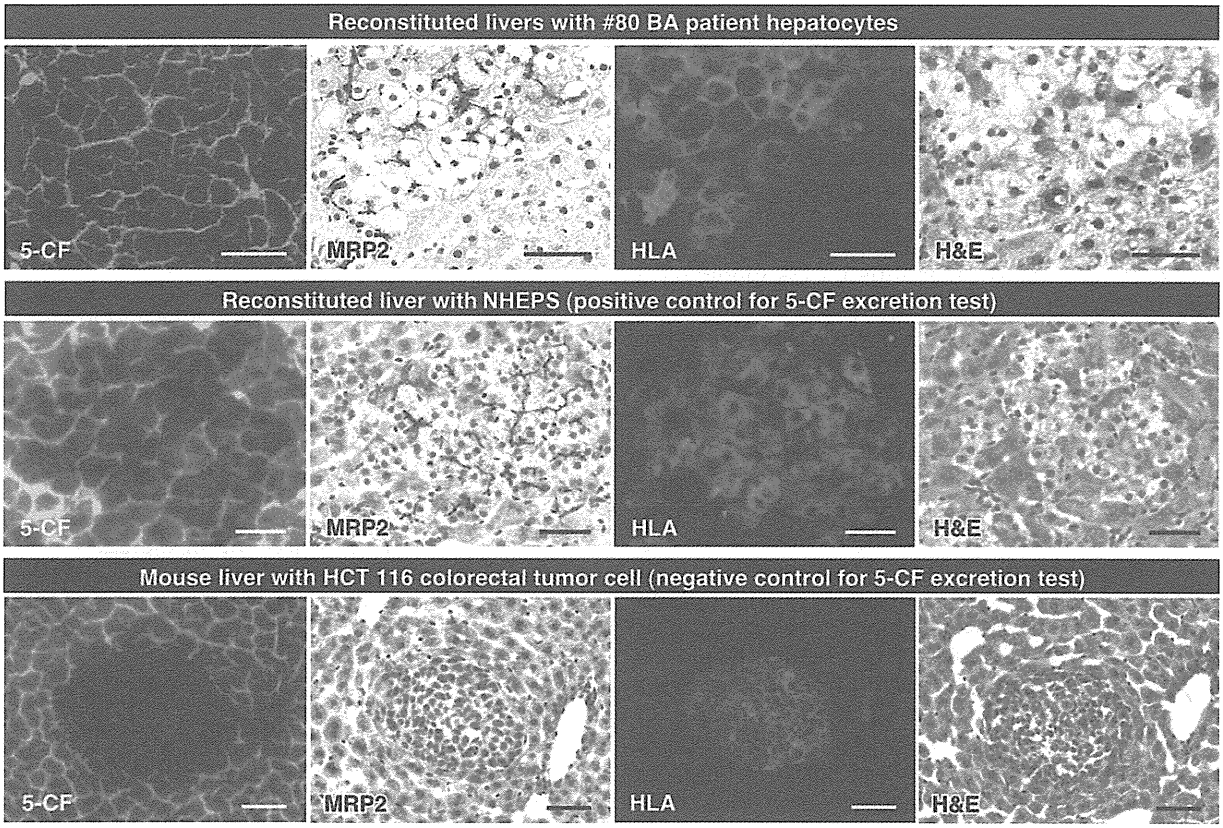

Figure 4. Functional integrity of the BC network within the reconstituted livers. Biliary excretion tests were performed with a fluorescent metabolic marker (5-CFDA). Serial sections were prepared from the livers of mice that received transplants of hepatocytes from a BA patient (patient 80), commercially available cryopreserved hepatocytes (NHEPS; positive control), or HCT 116 colorectal tumor cells (negative control). The sections were loaded with 5-CFDA, and the presence of the fluorescent metabolite 5-CF was assessed. In the livers reconstituted with patient hepatocytes and NHEPS hepatocytes, 5-CF (green on a dark field) was rapidly excreted into the BCs that formed the honeycomb networks over the lobule. In contrast, the BCs around the tumor, which formed after the transplantation of HCT 116 colorectal tumor cells, did not have this honeycomb pattern. Additional sections were stained for human MRP2 (brown in a bright field) and HLA (red in a dark field); H&E staining was also performed. The scale bars represent 50 μm.

and function. Bhogal et al.[29] reported that the viability, the total cell yield, and the success rate with cirrhotic tissues were low. In the current study, the cell yield and cell viability of hepatocytes from BA patients with fibrosis grade II or III were comparable to the yields and viability previously reported by Gramignoli et al. We expected that the low cell yield and viability would depend on the degree of fibrosis in patients with BA. However, there were no significant differences in the cell yields of hepatocytes from patients with grade II fibrosis and hepatocytes from patients with grade III fibrosis (Fig. 1C) or in cell viability (Fig. 1D). These results indicate that regardless of the extent of hepatic fibrosis, the presence of fibrosis affects the cell yield and viability when hepatocytes are isolated from the livers of patients with BA.

For hepatocytes from patient 80, 3 different conditions (freshly isolated, chilled, and frozen-thawed hepatocytes) were compared in terms of their engraftment and proliferative potential in a liver failure model using uPA-NOG mice. HLA-positive hepatocyte colonies were observed in the livers of all uPA-NOG mice that underwent transplantation with hepatocytes of any condition; however, a higher ratio of hALB-secreting mice and a higher level of serum hALB were observed in the mice that underwent transplantation with freshly isolated hepatocytes (Table 2). We succeeded in isolating a small number of hepatocytes buried in the severely cirrhotic liver of BA patient 149 (fibrosis grade III), and surprisingly, the hepatocytes could successfully engraft and proliferate within the uPA-NOG mouse livers as HLA-positive colonies. These results indicate that even hepatocytes buried in the cirrhotic livers of patients with BA do not lose their proliferative potential.

Recent studies of the molecular biology of BA have revealed no significant differences in the hepatic MRP2 expression levels of BA patients and control groups.[30] In fact, we confirmed the expression of not only the adenosine triphosphate–binding cassette, subfamily C (cystic fibrosis transmembrane conductance regulator (CFTR)/multidrug resistance-associated protein (MRP)), member 2 (ABCC2) gene but also the MRP2 protein, which was located on the apical plasma membranes of hepatocytes both in the livers of BA patients (Fig. 3A, left) and in partially humanized livers repopulated with hepatocytes from patients

with BA (Fig. 3B, left). Despite the normal MRP2 protein expression in the livers of patients with BA and in the partially humanized mouse liver, the bile was accumulated only in the many BCs of livers from patients with BA. This result clearly demonstrates the extrahepatic obstruction of the biliary flow.

In this study, using a reconstituted-liver mouse model, we examined the hepatocytes of patients with BA for the presence of abnormalities in vivo. Unfortunately, we failed to establish a BA model with liver-injured mice. However, this result indicates that the primary etiology of BA is absent in the hepatocytes themselves, and the hepatocytes buried in the cirrhotic livers of patients with BA are functionally intact hepatocytes retaining their proliferative potential and able to reconstitute a partially functioning human liver in mice. Gramignoli et al.[28] recently reported the isolation of hepatocytes from patients with many metabolic diseases, including BA, and the rapid and efficient repopulation of FRG (fumarylacetoacetate hydrolase (Fah), recombination activating gene 2 (Rag2) and interleukin 2 receptor gamma chain (Il-2rγ) triple gene knockout) mouse livers after the transplantation of hepatocytes obtained from patients with metabolic disease. In addition to Gramignoli et al.'s report, the current study supports the hypothesis that hepatocytes from patients with BA are morphologically and biochemically normal.

Recently, it has been reported that the extent of liver fibrosis at the time of portoenterostomy, as evaluated by picrosirius red staining, appears to be a strong negative predictor of outcomes.[31] The negative correlation between the extent of liver fibrosis and the yield of viable hepatocytes suggested by our results might be associated with that phenomenon. These results support the possibility that if the primary etiology is removed by Kasai portoenterostomy before progressive cholestasis develops, the liver of the patient with BA may regenerate autologously via the functionally intact hepatocytes remaining in the cirrhotic liver. The hepatocyte function in patients with BA may be independent of the degree of fibrosis; therefore, efforts to ameliorate the fibrosis would have great promise in treating this disease. Treatment would include an earlier diagnosis and surgery but might also include developing antifibrotic pharmacological approaches. If a method for earlier diagnosis or new drugs are developed in the near future, patients with BA may not require an operation that is as difficult as LT.

ACKNOWLEDGMENT

The authors thank M. Kuronuma, Y. Ando, T. Ogura, T. Kamisako, and R. Takahashi for their outstanding technical assistance with the animal experiments. They also thank M. Yamamoto, H. Nabekawa, and C. Kito for their technical assistance with molecular analyses and Dr. M. Ito, S. Enosawa, and M. Onodera for their helpful discussions.

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「小児とAYA世代の増殖性血液疾患の診断精度向上と
診療ガイドラインの改訂のための研究」

平成26年度

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