The degree of hepatic fibrosis by Ishak score [31] was 0 ± 0, 3.60 ± 0.43, and 1.67 ± 0.41 of the recipient liver tissues in control mice, nontransplanted CCl_4-treated mice, and SHED-transplanted CCl_4-treated mice, respectively (Fig. 2d). Colorimetric and real-time PCR assays revealed that SHED transplantation significantly reduced the hydroxyproline content and collagen production in the CCl_4-damaged liver tissues (Fig. 2e, f). Interestingly, HLA-ABC, hepatocyte paraffin 1, or human albumin-positive cells captured a similar area to the fibrous deposit region in the liver of nontransplanted CCl_4-treated mice (Fig. 1d–f). To confirm the in vivo hepatogenic differentiation capacity and therapeutic efficacy of SHED in recipient CCl_4-injured livers, we infused pediatric human gingival fibroblasts as a control for SHED transplantation in CCl_4-treated mice (Figure S6A in Additional file 2). Immunohistochemical assay showed that no HLA-ABC, hepatocyte paraffin 1, or human albumin-positive human cells were detected in the recipient CCl_4-damaged liver tissues (Figure S6B in Additional file 2). Biochemical assays demonstrated that human gingival fibroblast infusion did not recover the impaired hepatic function in CCl_4-injected mice (Figure S6C in Additional file 2). Taken together, these findings indicated that SHED transplantation suppressed CCl_4-enhanced fibrous deposition in the liver of CCl_4-treated mice, and suggested that SHED directly/spontaneously transdifferentiated into human hepatocytes in CCl_4-damaged livers.

Activation of hepatic stellate cells is a crucial event required to initiate and promote hepatic fibrosis, followed by producing and remodeling of type I collagen by matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinase (TIMPs) [34]. We therefore examined the kinetics of activated hepatic stellate cells after SHED transplantation in recipient livers 8 weeks after the first CCl_4 injection. Immunohistochemical analysis indicated that SHED transplantation decreased the area of alpha smooth muscle actin (αSMA)-positive cells, which indicated activated hepatic stellate cells, in the CCl_4-injured liver tissues (Fig. 3a, b). A real-time PCR assay also demonstrated that SHED transplantation significantly reduced the expression of αSMA mRNA (Fig. 3c) and markedly suppressed CCl_4-induced MMP2, MMP9, TIMP1, and TIMP2 mRNA expression (Fig. 3d) in the injured livers.

Kupffer cells and T lymphocytes and the fibrotic and inflammatory cytokines, such as TGF-β, TNFα, IL-6, and IL-17, produced by them are also involved in the progression of hepatic fibrosis and activation of hepatic stellate cells [34, 35]. By immunohistochemical assays, CCl_4 treatment markedly induced infiltration of F4/80-positive and CD3-positive cells in the liver, which indicate Kupffer cells and/or macrophages and T lymphocytes, respectively, compared with non-CCl_4-treated livers (Fig. 4a–c). SHED transplantation suppressed the altered distribution of F4/80-positive and CD3-positive cells in the CCl_4-treated livers (Fig. 4a–d). Further histochemical analysis demonstrated that SHED transplantation did not induce any heavy infiltration of lymphocyte-like cells, and did not cause any severe change of structural components in other tissues such as the kidneys, lungs, and spleens of CCl_4-treated mice with SHED (Figure S3A in Additional file 2). Real-time PCR and ELISA studies demonstrated that SHED transplantation reduced the expression of TGF-β1, TNFα, and IL-6 mRNAs in the CCl_4-induced fibrous livers (Fig. 3e), and suppressed the elevation of IL-6, TGF-β, and TNFα in the serum of CCl_4-treated mice (Fig. 4e). SHED transplantation reduced the proinflammatory IL-17 expression and recovered the decreased anti-inflammatory IL-10 expression in the CCl_4-treated livers (Fig. 4e). Taken together, these findings indicated that transplanted SHED might exhibit anti-fibrotic and anti-inflammatory effects against liver fibrosis by suppressing the activation of hepatic stellate cells, Kupffer cells/macrophages, and T cells.

Donor SHED are capable of differentiating into human hepatocyte-like cells without fusion in CCl_4-injured mouse livers

Transplanted bone marrow cells fuse with host hepatocytes in damaged livers [36, 37], but bone marrow MSCs differentiate into hepatocytes without cell fusion in recipients [24]. Using dual immunofluorescent staining using human specific antibodies to hepatocyte paraffin 1 and albumin, we demonstrated that double positive cells to hepatocyte paraffin 1 and human albumin were found in liver tissues of CCl_4-injured mice with SHED transplantation (Fig. 5a). However, it was unclear whether the double positive cells were fused with host cells or not; a possibility of cell fusion between donor SHED and recipient hepatocytes remained. To evaluate whether the in vivo converted SHED-derived human hepatocyte-like cells were fused with host hepatocytes, we isolated human cells from recipient livers of SHED-transplanted CCl_4-treated mice (Figure S7 in Additional file 2). Pan-liver cells were isolated from the recipient livers with the collagenase digestion method, and stained with anti-HLA-ABC antibody. The HLA-ABC-positive cells were magnetically sorted to collect separately from HLA-ABC-negative cells. Flow cytometric analysis confirmed that the HLA-ABC-positive fraction was 95.5 ± 4.43 % positive to HLA-ABC, but negative to mouse H-2Kb (Fig. 5b). Double positive cells were also not detected (Fig. 5b). On the other hand, the HLA-ABC-negative fraction was 96.3 ± 5.68 % positive to H-2Kb, but 0 % to HLA-ABC (data not shown). The HLA-ABC-positive cells maintained under EGF, FGF2, and HGF

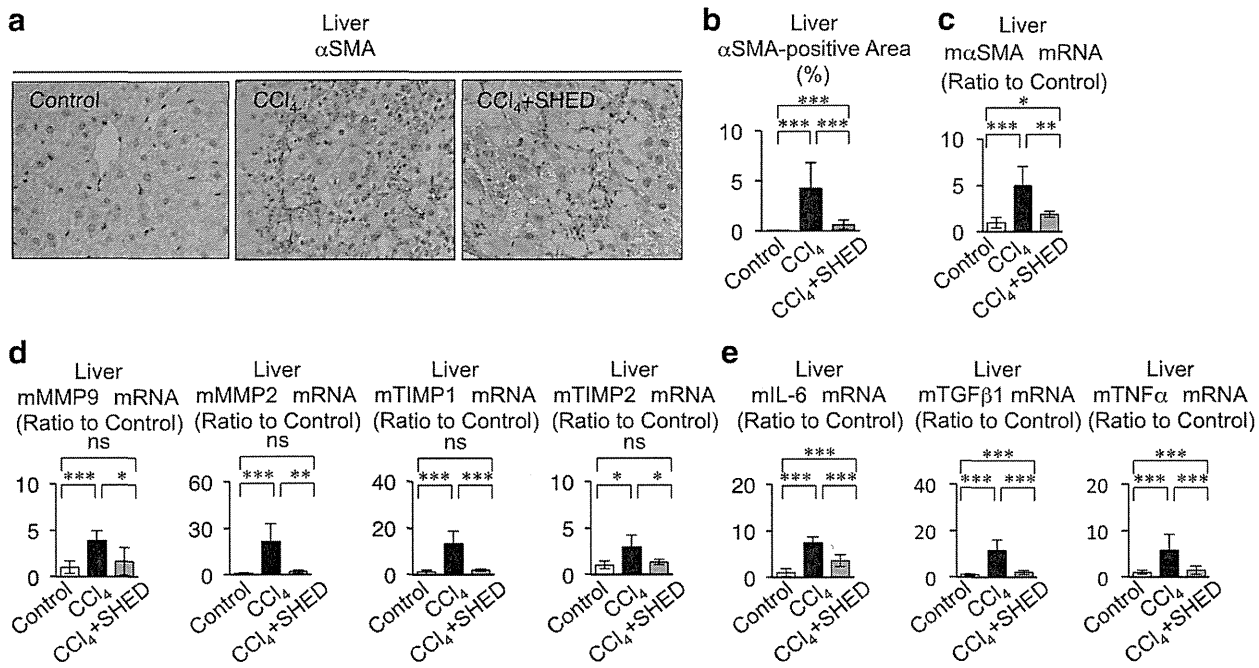

Fig. 3 SHED transplantation suppresses the activation of hepatic stellate cells in livers of CCl_4-treated mice. **a**–**c** Expression of alpha smooth muscle actin (*αSMA*). **a** Immunohistochemical staining with anti-αSMA antibody in recipient livers. **b** αSMA-positive area. **c** Real-time RT-PCR analysis of mouse αSMA (*mαSMA*) mRNA. **d**, **e** Real-time RT-PCR analysis. **d** Expression of mouse MMP9 and MMP2 (*mMMP9*, *mMMP2*) and mouse TIMP1 and TIMP2 (*mTIMP1*, *mTIMP2*) mRNA in recipient livers. **e** Expression of mouse interleukin-6 (*mIL-6*), mouse transforming growth factor β1 (*mTGFβ1*), and mouse tumor necrosis factor alpha(*mTNFα*) mRNA in recipient livers. **b**–**e** $n = 5$ for all groups. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.005$. *ns* no significance. Graph bars show the mean ± SD. Control, olive oil-injected group; CCl_4, CCl_4-treated group; CCl_4 + SHED: SHED-transplanted CCl_4-treated group. *CCl_4* carbon tetrachloride, *MMP* matrix metalloproteinase, *SHED* stem cells from human exfoliated deciduous teeth, *TIMP* tissue inhibitor of matrix metalloproteinase

stimulation for 3 days showed a cuboidal shape on the dishes by toluidine blue staining (Fig, 5c). A genomic DNA assay demonstrated that a human specific gene, *Alu*, was detected only in HLA-ABC-positive cells, but not in HLA-ABC-negative cells (Fig. 5d). On the other hand, a mouse specific gene, *mpf1*, was not detected in HLA-ABC-positive cells, but was found in HLA-ABC-negative cells (Fig. 5d). RT-PCR analysis also demonstrated that human albumin gene was detected only in HLA-ABC-positive cells, but not in HLA-ABC-negative cells, while mouse albumin gene was expressed in HLA-ABC-negative cells, but not in HLA-ABC-positive cells (Fig. 5e). These data indicate that transplanted SHED were directly transdifferentiated into human hepatocytes without fusion with recipient mouse hepatocytes.

Further RT-PCR assay demonstrated that the purified HLA-ABC-positive cells expressed human hepatocyte-specific genes, albumin, cytochrome P450 1A1, cytochrome P450 3A7, fumarylacetoacetase, tyrosine aminotransferase, uridine 5′-diphospho (UDP)-glucuronosyltransferase, transferrin, and transthyretin (Fig. 5f). However, the expression levels of human hepatocyte-specific genes in the purified HLA-ABC-positive cells were lower when compared with human hepatocyte cell line HepG2 (Fig. 5f). By ELISA and colorimetric assay, human albumin, urea, and blood urea nitrogen were detected at 4.8 ± 0.085 ng/ml, 0.47 ± 0.01 mg/dl, and 0.22 ± 0.005 mg/dl, respectively, in the culture supernatant of HLA-positive cells cultured with EGF, FGF2, and HGF stimulation for 3 days. Taken together, these findings indicate that SHED might show a potential for transdifferentiating into functional human hepatocytes, at least partially, without fusing with host mouse hepatocytes in fibrotic livers of CCl_4-treated mice.

Secondary transplantation of SHED-derived human hepatocyte-like cells purified from primary CCl_4-injured recipient livers recovered hepatic dysfunction of CCl_4-treated mice

Next we examined the homing capability of SHED-derived in vivo-converted hepatocyte-like cells. Mice that had been treated with CCl_4 for 4 weeks underwent secondary transplantation of purified HLA-ABC-positive cells (1×10^6), as well as HLA-ABC-negative cells (1×10^6), into the spleen (Fig. 6a). In vivo imaging analysis showed that strong intensity of DiR-labeled HLA-ABC-positive and DiR-labeled HLA-ABC-negative

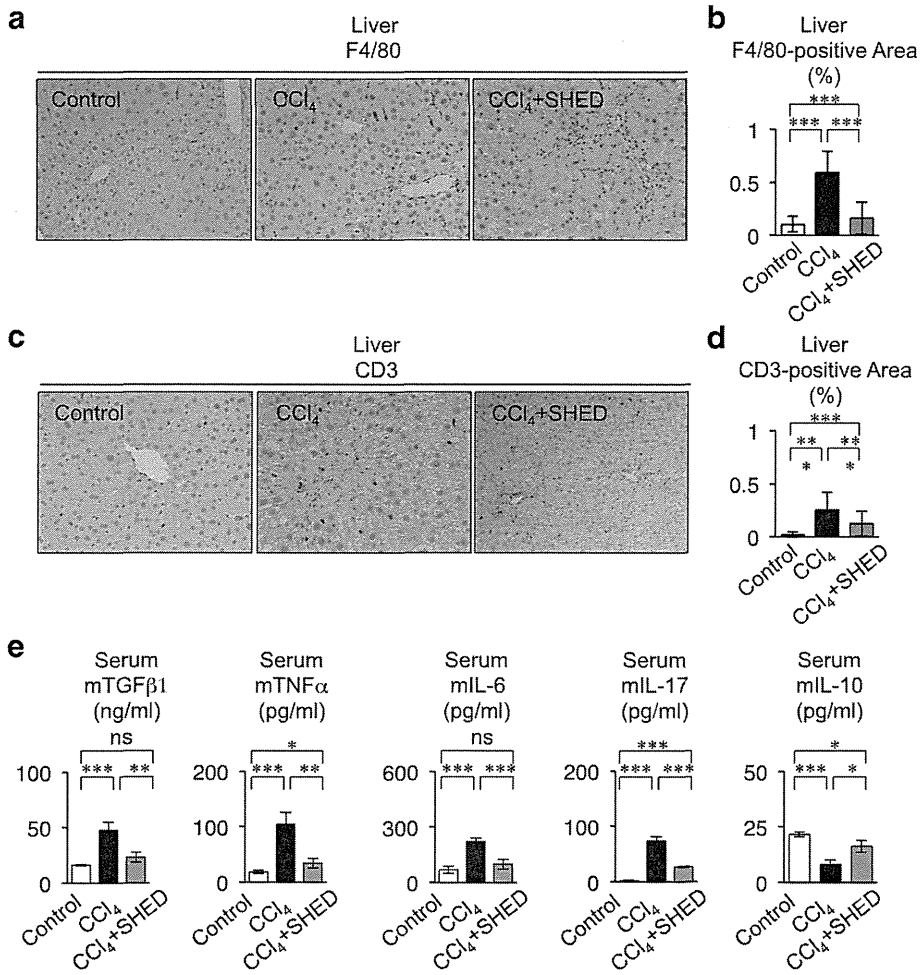

Fig. 4 SHED transplantation inhibits the enhanced distribution of Kupffer cells and T cells in recipient livers of CCl_4-treated mice. **a–c** Expression of F4/80 and CD3 in recipient livers. Immunohistochemical staining with anti-F4/80 (**a**) and anti-CD3 antibodies (**c**). The F4/80-positive (**b**) and CD3-positive (**d**) area. **e** ELISA of recipient serum. **b**, **d**, **e** $n = 5$ for all groups. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.005$. Graph bars show the means ± SD. Control, olive oil-injected control group; CCl_4, CCl_4-treated group; CCl_4 + SHED, SHED-transplanted CCl_4-treated group. *CCl_4* carbon tetrachloride, *mIL* mouse interleukin, *mTGFβ1* mouse transforming growth factor β1, *mTNFα* mouse tumor necrosis factor alpha, *SHED* stem cells from human exfoliated deciduous teeth

cells was observed in the livers of CCl_4-treated mice 24 hours post transplantation (Fig. 6b). Further immunohistochemical analysis and ELISA was performed in the liver tissues and peripheral blood serum of CCl_4-treated mice that underwent secondary transplantation with HLA-ABC-positive and HLA-ABC-negative cells, as well as of nontransplanted CCl_4-treated mice and nonCCl_4-treated mice, in week 8.

An immunohistochemical examination demonstrated that HLA-ABC-positive, hepatocyte paraffin 1-positive, and human albumin-positive cells were observed in the interlobular and portal regions corresponding to the fibular deposited area in liver tissues of CCl_4-treated mice that underwent secondary transplant with HLA-ABC-positive cells 4 weeks after the primary transplant (Fig. 6c). The HLA-ABC-positive, hepatocyte paraffin 1-positive, and human albumin-positive cell areas were 23.22 ± 6.81 %, 19.31 ± 5.06 %, and 17.80 ± 4.71 % in the secondary recipient livers (Fig. 6d). The immunohistochemically positive areas expressed a similar rate to the liver fibrous area of nontransplanted CCl_4-injured mice (Figure S8 in Additional file 2). No immunoreactivity against HLA-ABC, hepatocyte paraffin 1, or human albumin was detected in the liver tissues of CCl_4-induced mice that underwent secondary transplant with HLA-ABC-negative cells (Fig. 6c) or in nontransplanted CCl_4-induced mice and non-CCl_4-induced mice (data not shown). ELISA also showed that serum human albumin was detected in CCl_4-treated mice that underwent secondary transplant with HLA-ABC-positive cells, but not in CCl_4-treated mice that underwent secondary transplant with HLA-ABC-negative cells,

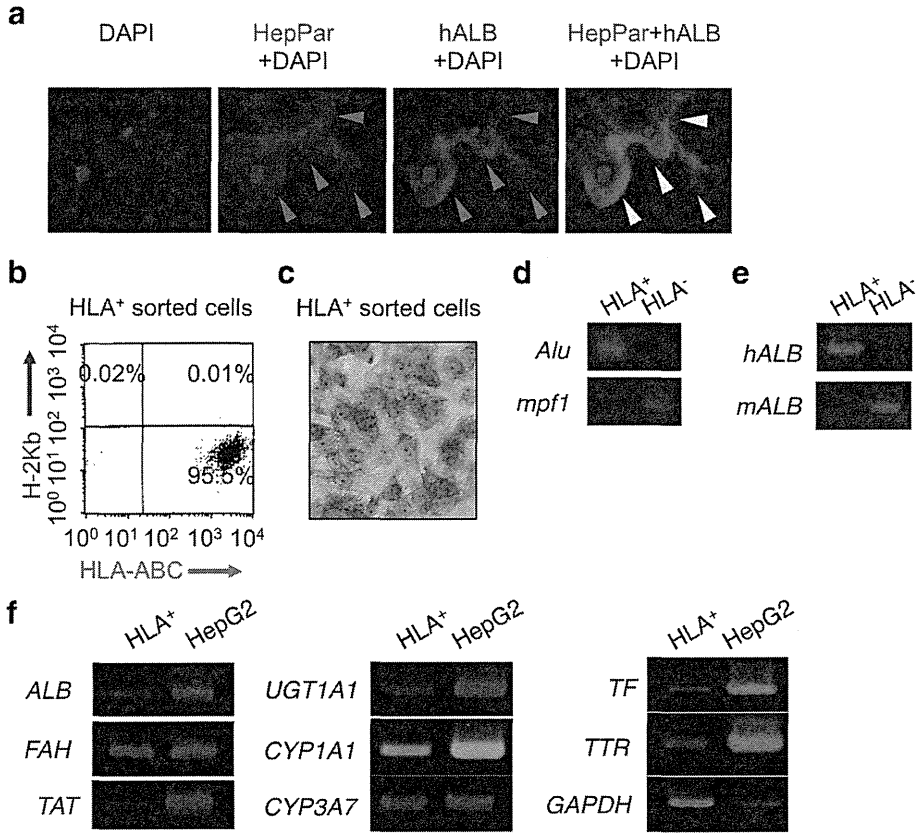

Fig. 5 SHED-derived HLA-ABC-positive cells purified from primary recipient livers of CCl_4-treated mice express hepatocyte-specific genes without host-cell fusion. **a** Double-immunofluorescent staining patterns for HepPar and human albumin (*hALB*) in CCl_4-injured liver tissues transplanted with SHED. **b** Flow cytometric analysis of magnetically sorted HLA-ABC-positive (*HLA*$^+$) cells stained with PE-conjugated anti-human HLA-ABC and APC-conjugated anti-mouse H-K2^b antibodies. **c** Morphology of sorted HLA$^+$ cells. Toluidine blue staining. **d** Genomic DNA assay. **e** RT-PCR analysis of hALB and mouse albumin (*mALB*) mRNAs. **f** RT-PCR analysis of human hepatocyte-specific genes. *ALB* albumin, *Alu* human-specific Alu gene, *CCl_4* carbon tetrachloride, *CYP1A1* cytochrome P450 1A1, *CYP3A7* cytochrome P450 3A7, *DAPI* 4′,6-diamidino-2-phenylindole, *FAH* fumarylacetoacetate hydrolase, *GAPDH* human glyceraldehyde 3-phosphate dehydrogenase, *HepG2* human hepatoma cell line, *HepPar1* human hepatocyte specific HepParaffin 1 antigen, *HLA* human leukocyte antigen, *HLA*$^-$ HLA-ABC-negative cells, *mpf1* mouse-specific Pf1 gene, *SHED* stem cells from human exfoliated deciduous teeth, *TAT* tyrosine aminotransferase, *TF* transferrin, *TTR* transthyretin, *UGT1A1* uridine 5′-diphospho-glucuronosyltransferase 1A1

nontransplanted CCl_4-treated mice, and non-CCl_4-treated mice (Fig. 6e).

To evaluate a therapeutic efficacy of SHED-derived in vivo-converted hepatocyte-like cells, peripheral blood serum and liver tissues were harvested from the mice in week 8. Serum assay demonstrated that the secondary transplantation of primary HLA-ABC-positive cells recovered hepatic markers of CCl_4-treated mice (Fig. 7a; Figure S9 in Additional file 2). Masson trichrome staining and hydroxyproline content assay demonstrated that the secondary transplantation of primary HLA-ABC-positive cells reduced the production and deposition of fibrous matrix (Fig. 7b–d; Figure S10A in Additional file 2). By real-time RT-PCR, expression of mouse type I collagen mRNA was also suppressed in the secondary recipient liver transplanted with HLA-ABC-positive cells compared with the nontransplanted recipient livers (Figure S10B in Additional file 2). On the other hand, the secondary transplantation of HLA-ABC-negative cells did not restore the hepatic function and fibrous tissue deposition in CCl_4-injured livers (Fig. 7; Figures S9 and S10 in Additional file 2).

Moreover, by immunohistochemical and real-time PCR analyses, we demonstrated that secondary transplantation of HLA-ABC-positive cells significantly reduced the increased αSMA expression in CCl_4-injured liver tissues (Fig. 8a–c). Further real-time PCR assay demonstrated that the secondary transplantation of HLA-ABC-positive cells markedly inhibited the enhanced MMP2, MMP9, TIMP1, and TIMP2 mRNA expressions (Fig. 8d) in CCl_4-injured livers. On the other hand, the increased distribution of αSMA-positive cells and enhanced expression of αSMA, MMP2, MMP9, TIMP1, TIMP2, TGF-β1, TNFα, and IL-6 mRNAs were not recovered in CCl_4-treated mice

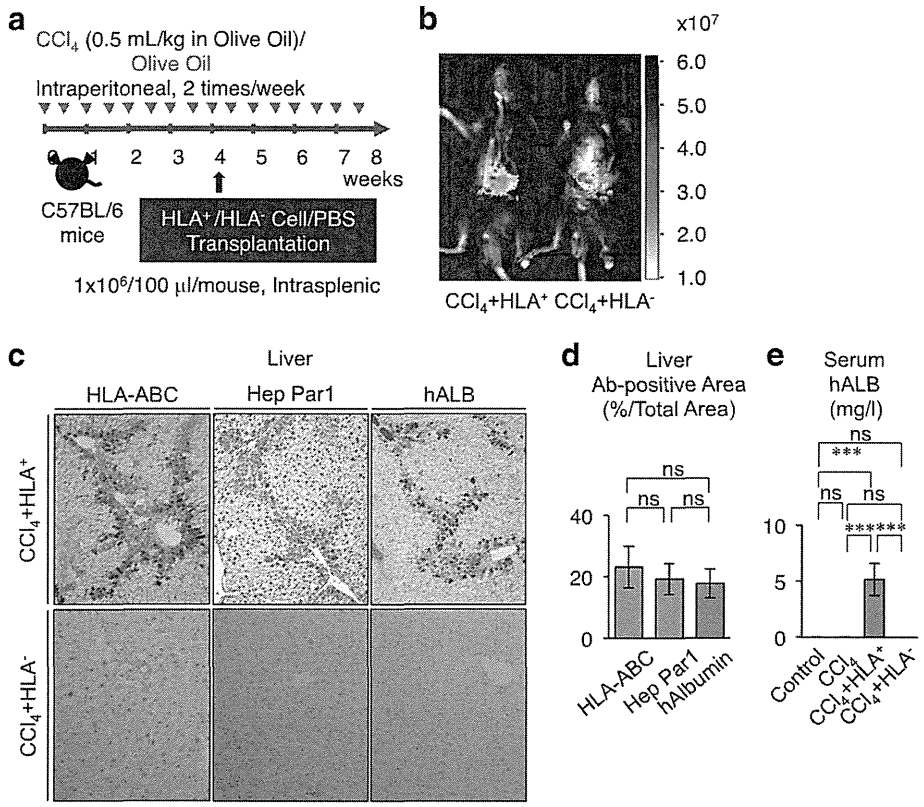

Fig. 6 Secondary transplanted primary HLA-ABC-positive cells home to CCl_4-treated recipient livers. **a** Schema of secondary transplantation of primary HLA-ABC$^+$/HLA-ABC$^-$ cells into C57BL/6 mice. HLA-ABC$^+$/HLA-ABC$^-$ cells (1×10^6) or phosphate-buffered saline (*PBS*) were infused into the mice that had intraperitoneally received CCl_4 (0.5 ml/kg) or olive oil only twice a week (*red arrows*). **b** In vivo monitoring of DiR-labeled HLA-ABC-positive (*HLA$^+$*) and HLA-negative (*HLA$^-$*) cells in CCl_4-treated mice 24 hours (24h) after the infusion. **c**, **d** Distribution of transplanted HLA$^+$ and HLA$^-$ cells in the secondary recipient livers. Immunohistochemistry with anti-human HLA-ABC (HLA-ABC), anti-hepatocyte paraffin 1 (Hep Par1), or anti-human albumin (hALB) antibody. Representative images. **c** Counterstaining with hematoxylin. The human HLA-ABC, hepatocyte paraffin 1, or human albumin-positive area. **d** Immunopositive area shown as the ratio to the total area. **e** ELISA of human albumin (*hALB*) in the recipient serum. **c–e** $n = 5$ for all groups. ***$P < 0.005$. *ns* no significance. Graph bars show the mean ± SD. Control, olive oil-injected group; CCl_4, CCl_4-treated group; CCl_4 + HLA$^+$, HLA$^+$ cell-transplanted CCl_4-treated group; CCl_4 + HLA$^-$, HLA$^-$ cell-transplanted CCl_4-treated group. *Ab* antibody, *CCl_4* carbon tetrachloride, *HepPar1* human hepatocyte specific HepParaffin 1 antigen, *HLA* human leukocyte antigen

that underwent secondary transplant with HLA-ABC-negative cells (Fig. 8). Taken together, these findings suggested that in vivo-generated hepatocyte-like cells in CCl_4-injured livers with SHED transplantation worked functionally, at least partially, as human hepatocytes to display therapeutic efficacy for CCl_4-induced liver fibrosis [38].

Discussion

Severe shortage of donor organs is a major challenge for liver transplantation [1]. Because of their unique capacities for homing and hepatic differentiation, MSCs and hematopoietic stem cells have been receiving attention as a source for cell therapy as an alternative to liver transplantation [39]. Transplantation of isolated mature hepatocytes has been used as an experimental therapy for liver disease in a limited number of cases. Recently, 100 cases of hepatocyte transplantation have been reported. Clinically, hepatocyte transplants express a proven efficiency, particularly in cases of metabolic liver disease where reversal or amelioration of the characteristic symptoms of the disease is easily quantified. However, no patients are completely corrected of a metabolic liver disease for a significant amount of time by hepatocyte transplantation alone [40]. MSC transplantation [12–14], as well as hematopoietic stem cell transplantation [41, 42], can successfully treat liver failure in animal models. MSCs exhibit a greater therapeutic efficacy with regard to homing and reducing fibrosis in comparison with hematopoietic stem cells in injured livers [43, 44]. In the present study, we demonstrated that SHED transplantation improved CCl_4-induced liver fibrosis and hepatic dysfunction via inertness of activated hepatic stellate cells and by replacement of damaged

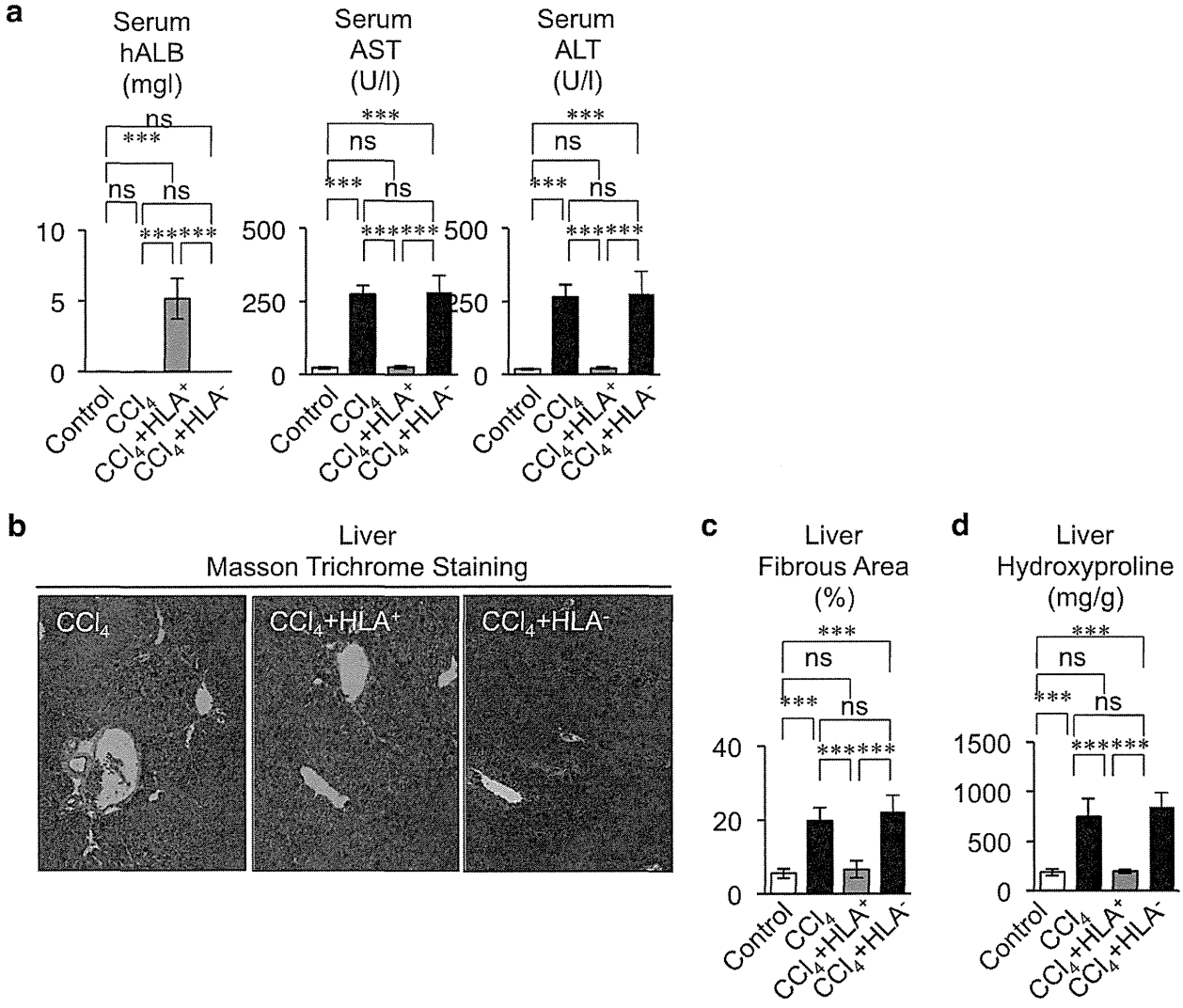

Fig. 7 Secondary transplantation of primary HLA-ABC-positive cells ameliorates the liver dysfunction in CCl_4-treated recipient livers. **a** Serum assays for the hepatic function. **b–d** Liver fibrosis assays. **b** Representative images of livers. Masson Trichrome staining. **c** Fibrotic area. **d** Hydroxyproline assay in recipient livers. **a, c, d** $n = 5$ for all groups. ***P <0.005. *ns* no significance. Graph bars show the mean ± SD. Control, olive oil-injected group; CCl_4, CCl_4-treated group; CCl_4 + HLA^+, HLA^+ cell-transplanted CCl_4-treated group; CCl_4 + HLA^-, HLA^- cell-transplanted CCl_4-treated group. *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *CCl_4* carbon tetrachloride, *hALB* human albumin, *HLA* human leukocyte antigen

tissue with transplanted SHED-derived hepatocyte-like cells. These findings therefore suggest that SHED might be a promising MSC source for liver regeneration.

The present study demonstrated that SHED transplantation markedly suppressed not only the pathological activation of hepatic stellate cells, but also the excessive infiltration of Kupffer cells and T cells in CCl_4-damaged mouse livers. Furthermore, SHED transplantation significantly reduced the enhanced production of fibrogenic and inflammatory factors, such as TGF-β1, TNFα, MMP2, MMP9, TIMP1, TIMP2, IL-6, and IL-17, and enhanced the expression of the anti-inflammatory factor IL-10 in CCl_4-induced fibrous livers. Activated hepatic stellate cells contribute to liver fibrosis via abnormal production of MMP2, TIMP1, and TIMP2 through the secretion of various inflammatory cytokines from Kupffer cells and T cells [34, 35]. SHED can induce Tregs and suppress Th17 cells and monocytes/dendritic cells [16, 17]. Transplanted SHED might therefore suppress immune responses and promote anti-fibrotic regulation by affecting hepatic stellate cells, Kupffer cells, and T cells in CCl_4-damaged mouse livers.

We speculate that a considerable number of transplanted SHED might be rejected immunologically owing to the present xenogeneic transplantation system and

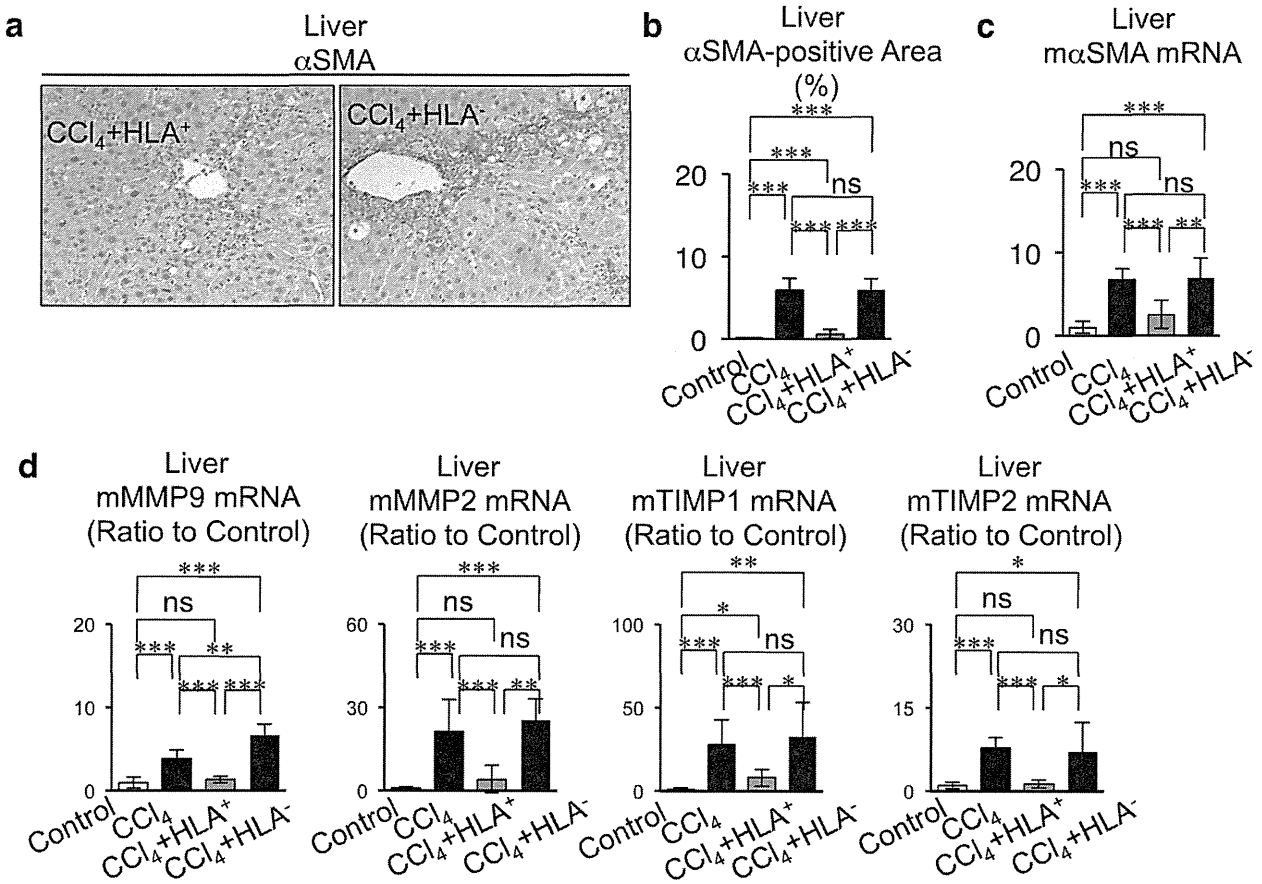

Fig. 8 Secondary transplantation of primary HLA-ABC-positive cells suppresses the activation of hepatic stellate cells and induction of Kupffer cells in livers CCl_4-treated mice. **a–c** Expression of alpha smooth muscle actin (*αSMA*). **a** Immunohistochemical staining with anti-αSMA antibody in recipient livers. **b** αSMA-positive area. **c** Real-time RT-PCR analysis of mouse αSMA (*mαSMA*) mRNA. **d** Real-time RT-PCR analysis of mouse MMP9 and MMP2 (*mMMP9*, *mMMP2*) and mouse TIMP1 and TIMP2 (*mTIMP1*, *mTIMP2*) mRNA in recipient livers. **b–d** $n = 5$ for all groups. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.005$. *ns* no significance. Graph bars show the mean ± SD. Control, olive oil-injected group; CCl_4, CCl_4-treated group; CCl_4 + HLA^+, HLA^+ cell-transplanted CCl_4-treated group; CCl_4 + HLA^-, HLA^- cell-transplanted CCl_4-treated group. *CCl_4* carbon tetrachloride, *HLA* human leukocyte antigen, *MMP* matrix metalloproteinase, *TIMP* tissue inhibitor of matrix metalloproteinase

nonimmunosuppressive status in immunocompetent mice. We also consider a possibility that donor SHED and the differentiated hepatocytes, as well as recipient hepatocytes, might be damaged by chronic CCl_4 stimuli. On the contrary, a result that donor SHED survived to differentiate into human hepatocytes in CCl_4-injured liver tissues suggests that the donor cells maintained higher toxic resistance compared with recipient cells, and supports that donor SHED, at least partially, showed a tolerance to host immune response, even under nonimmunosuppressive condition, in immunocompetent mice. Furthermore, SHED transplantation did not induce any heavy infiltration of lymphocyte-like cells, as well as any change of structural components, in other tissues including the kidney, lung, and spleen of CCl_4-treated mice. On the other hand, SHED transplantation suppressed the immune reaction in CCl_4-treated mice. These findings support that donor SHED did not cause any graft versus host disease-like reaction. Taken together, these findings suppose that SHED might exhibit safe immunology in the present xenogeneic transplantation system. Less HLA-DR expression and active immunomodulatory function of SHED may support a low immunogenicity and can acquire immune tolerance in vivo [16, 45]. Further study will be necessary to confirm the immunological safety of SHED as a donor for allogenic transplantation, as well as autologous transplantation, for liver patients.

The liver is a site of hematopoiesis in the fetus, so bone marrow hematopoietic stem cells have been considered an origin for hepatocytes in adults [46, 47]. Transplanted hematopoietic stem cells fuse with host hepatic cells to repopulate the liver as functional hepatocytes [36, 37]. On the other hand, a nonfusion origin of human hepatocytes was proposed in mouse liver

transplanted with human hematopoietic cells [48–50]. Engrafted bone marrow MSCs directly transdifferentiated into hepatocytes without cell fusion in rat livers [24]. Therefore, whether donor human cells fuse with recipient hepatic cells in mouse liver has not yet been fully understood. The presented three different approaches with a cell sorting technique of MHC class I antigen HLA-ABC-expressed human cells from the recipient mouse liver were carried out to evaluate the possibility of fusion between donor human MSCs and recipient murine hepatocytes. By flow cytometric analysis using human and mouse specific antibodies against MHC class I antigen, cell fusion of the donor cells and recipient cells was excluded. PCR analysis using human and mouse specific primers also omits the possibility of cell fusion. In a further secondary transplant assay, HLA-ABC-negative cells have in vivo differentiation capacity into human hepatocytes. These results indicate that donor-derived human hepatocytes have only human genetic and immunological properties, suggesting that cell fusion of donor SHED and recipient hepatocytes in the hepatogenic process may be a rare or nonexistent phenomenon in recipient CCl_4-injured mice. From another point of view, cell fusion between recipient hepatocytes and hematopoietic stem cells might lead to genetic instability and formation of cancer stem cells [51]. Human MSCs exhibit a low tumorigenic potential in vivo [52] and in vitro [53]. The present findings indicate that SHED may provide an attractive and safe source for stem cell-based liver regeneration. However, a long-term in vivo experiment will be necessary to assess the safety and tumorigenic risk(s) after SHED transplantation in damaged livers.

The present immunohistochemical findings suggest that intrasplenically infused donor SHED are transported into recipient liver through the portal vein system via the splenic vein, and penetrated into CCl_4-damaged fibrous area via the interlobular portal veins. However, the mechanism underlying in vivo homing and hepatic potential of transplanted MSCs, including SHED, remains unclear. In vivo homing and hepatic potential of MSCs might be regulated by a microenvironment of injured liver tissues. Liver contributes to a niche for hematopoietic stem cells in the fetus [54] and in patients with osteomyelofibrosis [55]. Hepatic stellate cells support hematopoiesis in fetal livers [56], and activated hepatic stellate cells release a factor associated with stem cell homing and migration, C-X-C motif chemokine 12 [57], and a factor promoting hepatocyte proliferation and differentiation, HGF [58]. In addition, hepatic stellate cells modulate a hepatogenic potential of bone marrow MSCs [59]. These previous studies suggest that activated hepatic stellate cells might function as a niche to modulate the homing and hepatic differentiation of transplanted MSCs. Further studies will be necessary to elucidate cellular and molecular mechanism(s) responsible for in vivo homing and hepatic potential of transplanted MSCs, including SHED.

In this study, purified HLA-ABC-positive cells from liver tissue of SHED-transplanted CCl_4-treated mice confirmed the expression of several characteristics as human hepatocyte-like cells. The present secondary transplantation into CCl_4-treated mice analysis demonstrates that purified HLA-ABC-positive cells express a homing capacity and a treatment efficacy in CCl_4-injured mice, suggesting that in vivo-converted SHED-derived hepatocytes may function as human hepatocytes. Chimeric human livers with more than 90 % human hepatocytes are successfully developed in murine models [60, 61]. A recently reported novel tissue engineering approach generated a transplantable recellularized liver graft with human hepatocytes and MSCs using xenogeneic decellularized livers [62, 63]. The present in vivo serial transplantation assay demonstrated that SHED-derived direct-converted hepatocytes exhibit chimerism and therapeutic effect in CCl_4-damaged mouse livers. These results suggest that in vivo-generated human hepatocyte-like cells derived from donor SHED may provide an alternative source for banking of human hepatocytes and development of human chimeric livers in vivo *and* ex vivo.

Conclusion

In summary, this report provides a foundation for SHED-based liver regenerative medicine. Further studies will be required to elucidate whether this practical and unique approach can be applied clinically for patients with liver disorders, such as liver fibrosis, metabolic diseases, or some coagulopathies.

Additional files

Additional file 1: Presents supplementary methods. (DOC 91 kb)

Additional file 2: Figure S1. Showing characterization of SHED, **Figure S2** showing CFSE-labeled cell tracing of donor SHED 1 day after transplantation, **Figure S3** showing histological and immunohistochemical analyses of the kidney, lung, and spleen of CCl_4-treated mice with splenic SHED transplantation, **Figure S4** showing immunohistochemical negative control analysis, **Figure S5** showing immunohistochemical analysis of human liver, **Figure S6** showing transplantation of human gingival fibroblasts into CCl_4-treated mice, **Figure S7** showing the schema for cell sorting of human cells from recipient liver tissues of CCl_4-treated mice transplanted with SHED, **Figure S8** showing human HLA-ABC, hepatocyte paraffin1 (Hep Par1), or human albumin (hALB) antibody-positive area in the recipient liver tissues of secondary transplant CCL-treated mice, **Figure S9** showing serum assays for the hepatic function in secondary transplant CCl4-treated mice, and **Figure S10** showing fibrous assay in recipient livers of secondary transplant CCl4-treated mice. (PDF 1386 kb)

Additional file 3: Table S1. Presenting the TaqMan primers and probes used for mouse genes used in real-time PCR, and **Table S2** presenting the primer pairs used for human and mouse genes for genomic PCR and RT-PCR. (DOC 65 kb)

Abbreviations
ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; APC: Allophycocyanin; AST: Aspartate aminotransferase; CCl_4: Carbon tetrachloride; CFSE: Carboxyfluorescein diacetate succinimidyl ester; DiR: 1,1-Dioctadecyl-3,3,3,3-tetramethylindotricarbocyanine iodide; EGF: Epidermal growth factor; ELISA: Enzyme-linked immunosorbent assay; FGF2: Fibroblast growth factor 2; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; HGF: Hepatocyte growth factor; HLA: Human leukocyte antigen; IL: Interleukin; MHC: Major histocompatibility complex; MMP: Matrix metalloproteinase; MSC: Mesenchymal stem cell; NIR: Near infrared; P3: Passage 3; PBS: Phosphate-buffered saline; PE: Phycoerythrin; SD: Standard deviation; SHED: Stem cells from human exfoliated deciduous teeth; TGF-β1: Transforming growth factor β1; Th17: Interleukin-17-producing helper T; TIMP: Tissue inhibitor of metalloproteinase; TNFα: Tumor necrosis factor alpha; Tregs: Regulatory T cells; UDP: Uridine 5′-diphosphate; UDP: Uridine 5′-diphosphate; αSMA: Alpha smooth muscle actin.

Competing interests
The authors declare that they have no competing interest.

Authors' contributions
TY carried out conception and design of the study, generation, collection and assembly of data, interpretation of data, statistical analysis, and drafting of the manuscript. FSA carried out generation, collection and assembly of data, and interpretation of data. RY, HY, JKF, YY, KY, MH, TM, and RA participated in interpretation of data. KI, SO, SS, and KN participated in conception and design of the study, interpretation of data, and study supervision. TT participated in conception and design of the study, interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, and study supervision. All authors read and approved the final manuscript.

Acknowledgements
The authors are very thankful to Mr Brian Quinn for his English assistance with writing. They also appreciate Ms Tomoko Yamazaki (Department of Pediatric Surgery, Kyushu University Graduate School of Medical Sciences) for her excellent assistance and Dr Soichiro Sonoda (Department of Molecular Cell Biology and Oral Anatomy, Kyushu University Graduate School of Dental Science) for his histological assistance and writing support during this study. This work was supported by grants from the Japan Society for the Promotion of Science, including Grant-in-Aid for Scientific Research (A) (grant number 25253094 to TT), Grant-in-Aid for Scientific Research (B) (grant number 25293405 to TY), and Grant-in-Aid for Challenging Exploratory Research Project (grant numbers 23659618 and 25670744 to TT and grant number 24659815 to TY), from the Ministry of Education, Culture, Sports, Science and Technology of Japan for Translational Research Grant of Center for Clinical and Translational Research Seeds B3 to TT, and from the Ministry of Health, Labor and Welfare for Research on Rare and Intractable Diseases (grant number H26-040) to TT. This research is also partially supported by the Translational Research Network Program from Japan Agency for Medical Research and Development, AMED. The authors appreciate Professor Fusanori Nishimura (Department of Periodontology, Kyushu University Graduate School of Dental Science) for his technical assistance through the program for the Promotion of Strategic International Research Network accelerating Brain Rotation (S2605) by the Japan Society for the Promotion of Science.

Author details
[1]Department of Molecular Cell Biology and Oral Anatomy, Kyushu University Graduate School of Dental Science, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. [2]Department of Pediatric Surgery, Kyushu University Graduate School of Medical Sciences, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. [3]Department of Pediatric Dentistry, Kyushu University Graduate School of Dental Science, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. [4]Department of Pediatrics, Kyushu University Graduate School of Medical Sciences, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. [5]Department of Pediatrics, Faculty of Medicine, Oita University, 1-1 Idaigaoka, Hazama-cho, Yuhuin 879-5593, Japan. [6]Department of Pediatrics, Faculty of Medicine and Health Sciences, Yamaguchi University, 1-1-1 Minami Ogushi, Ube 755-8505, Japan. [7]Department Anatomy and Cell Biology, School of Dental Medicine, University of Pennsylvania, 240 South 40th Street, Philadelphia, PA 19104-6030, USA.

Received: 16 April 2015 Revised: 2 July 2015 Accepted: 12 August 2015
Published online: 10 September 2015

References
1. Murray KF, Carithers Jr RL. AASLD practice guidelines: evaluation of the patient for liver transplantation. Hepatology. 2005;41:1407–32.
2. Horslen SP, McCowan TC, Goertzen TC, Warkentin PI, Cai HB, Strom SC, et al. Isolated hepatocyte transplantation in an infant with a severe urea cycle disorder. Pediatrics. 2003;111:1262–7.
3. Ishikawa T, Banas A, Teratani T, Iwaguro H, Ochiya T. Regenerative cells for transplantation in hepatic failure. Cell Transplant. 2012;21:387–99.
4. Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, et al. Bone marrow as a potential source of hepatic oval cells. Science. 1999;284:1168–70.
5. Friedenstein AJ, Deriglasova UF, Kulagina NN, Panasuk AF, Rudakowa SF, Luriá EA, et al. Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method. Exp Hematol. 1974;22:83–92.
6. Katz AJ, Tholpady A, Tholpady SS, Shang H, Ogle RC. Cell surface and transcriptional characterization of human adipose-derived adherent stromal (hADAS) cells. Stem Cells. 2005;23:412–23.
7. Mareschi K, Biasin E, Piacibello W, Aglietta M, Madon E, Fagioli F. Isolation of human mesenchymal stem cells: bone marrow versus umbilical cord blood. Haematologica. 2001;86:1099–100.
8. Peng SY, Chou CJ, Cheng PJ, Ko IC, Kao YJ, Chen YH, et al. Therapeutic potential of amniotic-fluid-derived stem cells on liver fibrosis model in mice. Taiwan J Obstet Gynecol. 2014;53:151–7.
9. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci USA. 2000;97:13625–30.
10. Uccelli A, Pistoia V, Moretta L. Mesenchymal stem cells: a new strategy for immunosuppression? Trends Immunol. 2007;28:219–26.
11. Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J Cell Physiol. 2007;213:341–7.
12. Mohamadnejad M, Alimoghaddam K, Mohyeddin-Bonab M, Bagheri M, Bashtar M, Ghanaati H, et al. Phase 1 trial of autologous bone marrow mesenchymal stem cell transplantation in patients with decompensated liver cirrhosis. Arch Iran Med. 2007;10:459–66.
13. Kharaziha P, Hellström PM, Noorinayer B, Farzaneh F, Aghajani K, Jafari F, et al. Improvement of liver function in liver cirrhosis patients after autologous mesenchymal stem cell injection: a phase I-II clinical trial. Eur J Gastroenterol Hepatol. 2009;21:1199–205.
14. El-Ansary M, Abdel-Aziz I, Mogawer S, Abdel-Hamid S, Hammam O, Teaema S, et al. Phase II trial: undifferentiated versus differentiated autologous mesenchymal stem cells transplantation in Egyptian patients with HCV induced liver cirrhosis. Stem Cell Rev. 2012;8:972–81.
15. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci USA. 2003;100:5807–12.
16. Yamaza T, Kentaro A, Chen C, Liu Y, Shi Y, Gronthos S, et al. Immunomodulatory properties of stem cells from human exfoliated deciduous teeth. Stem Cell Res Ther. 2010;1:5.
17. Silva Fde S, Ramos RN, Almeida DC, Bassi EJ, Gonzales RP, Miyagi SP, et al. Mesenchymal stem cells derived from human exfoliated deciduous teeth (SHEDs) induce immune modulatory profile in monocyte-derived dendritic cells. PLoS One. 2014;9:e98050.
18. Seo BM, Sonoyama W, Yamaza T, Coppe C, Kikuiri T, Akiyama K, et al. SHED repair critical-size calvarial defects in immunocompromised mice. Oral Dis. 2008;14:428–34.
19. Zheng Y, Liu Y, Zhang CM, Zhang HY, Li WH, Shi S, et al. Stem cells from deciduous tooth repair mandibular defect in swine. J Dent Res. 2009;88:249–54.
20. Wang J, Wang X, Sun Z, Wang X, Yang H, Shi S, et al. Stem cells from human-exfoliated deciduous teeth can differentiate into dopaminergic neuron-like cells. Stem Cells Dev. 2010;19:1375–83.
21. Sakai K, Yamamoto A, Matsubara K, Nakamura S, Naruse M, Yamagata M, et al. Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. J Clin Invest. 2012;122:80–90.
22. Kanafi MM, Ramesh A, Gupta PK, Bhonde RR. Influence of hypoxia, high glucose, and low serum on the growth kinetics of mesenchymal stem cells from deciduous and permanent teeth. Cells Tissues Organs. 2013;198:198–208.

23. Ma L, Makino Y, Yamaza H, Akiyama K, Hoshino Y, Song G, et al. Cryopreserved dental pulp tissues of exfoliated deciduous teeth is a feasible stem cell resource for regenerative medicine. PLoS One. 2012;7:e51777.
24. Sato Y, Araki H, Kato J, Nakamura K, Kawano Y, Kobune M, et al. Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion. Blood. 2005;106:756–63.
25. Seo MJ, Suh SY, Bae YC, Jung JS. Differentiation of human adipose stromal cells into hepatic lineage in vitro and in vivo. Biochem Biophys Res Commun. 2005;328:258–64.
26. Jung KH, Shin HP, Lee S, Lim YJ, Hwang SH, Han H, et al. Effect of human umbilical cord blood-derived mesenchymal stem cells in a cirrhotic rat model. Liver Int. 2009;29:898–909.
27. Bishi DK, Mathapati S, Venugopal JR, Guhathakurta S, Cherian KM, Ramakrishna S, et al. Trans-differentiation of human mesenchymal stem cells generates functional hepatospheres on poly(L-lactic acid)-co-poly(ε-caprolactone)/collagen nanofibrous scaffolds. J Mater Chem B. 2013;1:3972–84.
28. Bishi DK, Mathapati S, Cherian KM, Guhathakurta S, Verma RS. In vitro hepatic trans-differentiation of human mesenchymal stem cells using sera from congestive/ischemic liver during cardiac failure. PLoS One. 2014;9:e92397.
29. Arora V, Arora P, Munshi AK. Banking stem cells from human exfoliated deciduous teeth (SHED): saving for the future. J Clin Pediatr Dent. 2009;33:289–94.
30. Ji EH, Song JS, Kim SO, Jeon M, Choi BJ, Lee JH. Viability of pulp stromal cells in cryopreserved deciduous teeth. Cell Tissue Bank. 2014;15:67–74.
31. Ishkitiev N, Yaegaki K, Calenic B, Nakahara T, Ishikawa H, Mitiev V, et al. Deciduous and permanent dental pulp mesenchymal cells acquire hepatic morphologic and functional features in vitro. J Endod. 2010;36:469–74.
32. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8:315–7.
33. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol. 1995;22:696–9.
34. Moreira RK. Hepatic Stellate Cells and liver fibrosis. Arch Pathol Lab Med. 2007;131:1728–34.
35. Meng F, Wang K, Aoyama T, Grivennikov SI, Paik Y, Scholten D, et al. Interleukin-17 signaling in inflammatory, Kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. Gastroenterology. 2012;143:765–76.
36. Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimy M, et al. Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature. 2003;422:897–901.
37. Vassilopoulos G, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. Nature. 2003;422:901–4.
38. Asgari S, Moslem M, Bagheri-Lankarani K, Pournasr B, Miryounesi M, Baharvand H. Differentiation and transplantation of human induced pluripotent stem cell-derived hepatocyte-like cells. Stem Cell Rev. 2013;9:493–504.
39. Kakinuma S, Nakauchi H, Watanabe M. Hepatic stem/progenitor cells and stem-cell transplantation for the treatment of liver disease. J Gastroentel. 2009;44:167–72.
40. Hansel MC, Gramignoli R, Skvorak KJ, Marongiu F, Bkake W, Davila J, et al. The history and use of human hepatocytes for the treatment of liver diseases: the first 100 patients. Curr Protoc Toxicol. 2014;62:14. 12.1–23.
41. Terai S, Ishikawa T, Omori K, Aoyama K, Marumoto Y, Urata Y, et al. Improved liver function in patients with liver cirrhosis after autologous bone marrow cell infusion therapy. Stem Cells. 2006;24:2292–8.
42. Salama H, Zekri AR, Zern M, Bahnassy A, Loutfy S, Shalaby S, et al. Autologous hematopoietic stem cell transplantation in 48 patients with end-stage chronic liver diseases. Cell Transplant. 2010;19:1475–86.
43. Pulavendran S, Vignesh J, Rose C. Differential anti-inflammatory and anti-fibrotic activity of transplanted mesenchymal vs. hematopoietic stem cells in carbon tetrachloride-induced liver injury in mice. Int Immunopharmacol. 2010;10:513–9.
44. Li Q, Zhou X, Shi Y, Li J, Zheng L, Cui L, et al. In vivo tracking and comparison of the therapeutic effects of MSCs and HSCs for liver injury. PLoS One. 2013;8:e62363.
45. Bernardi L, Luisi SB, Fernandes R, Dalberto TP, Valentim L, Bogo Chies JA, et al. The isolation of stem cells from human deciduous teeth pulp is related to the physiological process of resorption. J Endod. 2011;37:973–9.
46. Alison MR, Poulsom R, Jeffery R, Dhillon AP, Quaglia A, Jacob J, et al. Hepatocytes from non-hepatic adult stem cells. Nature. 2000;406:257.
47. Theise ND, Nimmakayalu M, Gardner R, Illei PB, Morgan G, Teperman L, et al. Liver from bone marrow in humans. Hepatology. 2000;32:11–6.
48. Jang YY, Collector MI, Baylin SB, Diehl AM, Sharkis SJ. Hematopoietic stem cells convert into liver cells within days without fusion. Nat Cell Biol. 2004;6:532–9.
49. Newsome PN, Johannessen I, Boyle S, Dalakas E, McAulay KA, Samuel K, et al. Human cord blood-derived cells can differentiate into hepatocytes in the mouse liver with no evidence of cellular fusion. Gastroenterology. 2003;124:1891–900.
50. Ishikawa F, Drake CJ, Yang S, Fleming P, Minamiguchi H, Visconti RP, et al. Transplanted human cord blood cells give rise to hepatocytes in engrafted mice. Ann N Y Acad Sci. 2003;996:174–85.
51. Bjerkvig R, Tysnes BB, Aboody KS, Najbauer J, Terzis AJ. Opinion: the origin of the cancer stem cell: current controversies and new insights. Nat Rev Cancer. 2005;5:899–904.
52. Miura M, Miura Y, Padilla-Nash HM, Molinolo AA, Fu B, Patel V, et al. Accumulated chromosomal instability in murine bone marrow mesenchymal stem cells leads to malignant transformation. Stem Cells. 2006;24:1095–103.
53. Kim J, Kang JW, Park JH, Choi Y, Choi KS, Park KD, et al. Biological characterization of long-term cultured human mesenchymal stem cells. Arch Pharm Res. 2009;32:117–26.
54. Jordan CT, McKearn JP, Lemischka IR. Cellular and developmental properties of fetal hematopoietic stem cells. Cell. 1990;61:953–63.
55. Kim CH. Homeostatic and pathogenic extramedullary hematopoiesis. J Blood Med. 2010;1:13–9.
56. Kordes C, Sawitza I, Götze S, Häussinger D. Hepatic stellate cells support hematopoiesis and are liver-resident mesenchymal stem cells. Cell Physiol Biochem. 2013;31:290–304.
57. Hong F, Tuyama A, Lee TF, Loke J, Agarwal R, Cheng X, et al. Hepatic stellate cells express functional CXCR4: role in stromal cell-derived factor-1alpha-mediated stellate cell activation. Hepatology. 2009;49:2055–67.
58. Ishikawa T, Factor VM, Marquardt JU, Raggi C, Seo D, Kitade M, et al. Hepatocyte growth factor/c-met signaling is required for stem-cell-mediated liver regeneration in mice. Hepatology. 2012;55:1215–26.
59. Deng X, Chen YX, Zhang X, Zhang JP, Yin C, Yue HY, et al. Hepatic stellate cells modulate the differentiation of bone marrow mesenchymal stem cells into hepatocyte-like cells. J Cell Physiol. 2008;217:138–44.
60. Azuma H, Paulk N, Ranade A, Dorrell C, Al-Dhalimy M, Ellis E, et al. Robust expansion of human hepatocytes in $Fah^{-/-}/Rag2^{-/-}/Il2rg^{-/-}$ mice. Nat Biotechnol. 2007;25:903–10.
61. Hasegawa M, Kawai K, Mitsui T, Taniguchi K, Monnai M, Wakui M, et al. The reconstituted 'humanized liver' in TK-NOG mice is mature and functional. Biochem Biophys Res Commun. 2011;405:405–10.
62. Uygun BE, Soto-Gutierrez A, Yagi H, Izamis ML, Guzzardi MA, Shulman C, et al. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. Nat Med. 2010;16:814–20.
63. Ji R, Zhang N, You N, Li Q, Liu W, Jiang N, et al. The differentiation of MSCs into functional hepatocyte-like cells in a liver biomatrix scaffold and their transplantation into liver-fibrotic mice. Biomaterials. 2012;33:8995–9008.

【長期予後と成人後の医学的問題】

小児外科疾患

八木　實[*1]　尾花和子[*2]　田口智章[*3]　仁尾正記[*4]

キーワード●小児外科，長期予後，トランジション

はじめに

日本における小児外科は，この半世紀で格段の進歩が認められている．平成26年，日本小児外科学会は設立50周年を迎えた．この50年間で新生児外科疾患，胆道閉鎖症，小児固形がんを中心に，救命の段階からQOLの向上も含めた長期生存の段階になり，昨今では小児期に手術を受けた患児の大部分が成人に達し天寿を全うする時代になっている．したがって現在，小児期に手術を受け，長期にわたって治療やフォローアップが必要な患児は，思春期や青年期になっても小児外科医が窓口になって，それぞれの臓器に特異的な診療科である耳鼻科，泌尿器科，産婦人科や成人内科と連携しながら診療している場合が多いのが現状である．

本稿では，小児外科各疾患の長期予後の概略と，それらの成人後の諸問題をトランジションの観点から概説したい．

I 小児外科疾患の長期予後

1. 新生児外科疾患の長期予後

新生児外科疾患は新生児期の手術のみで治癒する疾患が大部分であるが，術後長期にわたり治療やフォローアップを要するものもあり，成人期に達してもフォローアップしなければならないことも少なくない．先天性腸閉鎖症，臍帯ヘルニアや腹壁破裂などの腹壁異常，横隔膜ヘルニアなどで開腹手術を受けた患児は，通常は特に問題ないが，長期経過時に癒着性イレウスを起こすリスクを有していることは言うまでもない．したがって，成人期に至っても腹部に小児期に受けた術創がある場合は，成人期の術後と同様に，絞扼性イレウスの危険性も念頭に置く必要がある．

水腎症を含む閉塞性尿路疾患遠隔期例では，腎機能障害が進行し透析や腎移植につながるケースもあるので，腎臓内科医との連携も重要である．このほか，新生児外科疾患で術後も長期的なケアを必要とするものは，直腸肛門奇形（鎖肛），特に総排泄腔遺残症や高位鎖肛，総排泄腔外反症，食道閉鎖症の気管軟化症合併例，横隔膜ヘルニアの肺低形成例などである．

直腸肛門奇形（鎖肛）では骨盤底筋群の発育の悪い高位鎖肛例などを中心に，便失禁や高度な便秘などの排便障害や尿失禁などの排尿障害

Long term prognosis and transition in the post-operative patients of pediatric surgical field.

[*1]Minoru Yagi : Department of Pediatric Surgery, Kurume University School of Medicine, [*2]Kazuko Obana : Department of Pediatric Surgery, Yamanashi Prefectural Central Hospital, [*3]Tomoaki Taguchi : Department of Pediatric Surgery, Kyusyu University Graduate School of Medical Sciences, [*4]Masaki Nio : Department of Pediatric Surgery, Tohoku University Graduate School of Medicine

[*1]久留米大学医学部主任教授（小児外科），日本小児外科学会理事（トランジション検討委員会担当），[*2]山梨県立中央病院小児外科主任医長，日本小児外科学会トランジション検討委員会委員長，[*3]九州大学大学院医学研究院教授（小児外科），日本小児外科学会前理事長，[*4]東北大学大学院医学系研究科教授（小児外科），日本小児外科学会理事長

が継続する場合がある．総排泄腔遺残症および外反症では，排便障害のほかに，月経障害や妊娠困難など生殖器障害や難治性の尿路感染など泌尿器障害を併存することも多い．内科医のみならず産婦人科医や泌尿器科医との連携が必要で，ストーマ症例では皮膚・排泄ケアに関し認定を受けたWOC（wound, ostomy and continence）ナースや，こころのケアも含めたチームによる診療が必要であることも多い．

ヒルシュスプルング病の治療は，現在ではtransanal endorectal pull-through法が主流となり，術後良好な排便機能を認めることが多いが，過去においては術式が多岐にわたり，成人期になっても術後合併症由来の失禁，汚染，便秘，排尿障害が認められる症例も散見され，術後長期にわたって小児外科にとどまらず泌尿器科との連携でフォローアップを行うことが肝要である．

食道閉鎖症では嚥下障害や胃食道逆流症や気管軟化症のために呼吸困難や肺炎を起こすことがある．特に上気道感染を契機に症状が悪化することもあるので注意を要する．小児期から耳鼻科医との連携を図ることも重要で，成人期例では内科医や耳鼻科医との連携が必要である．最近では胸筋を切開せずに低侵襲で手術が行われているが，以前の症例では胸筋切開に由来する側弯症例が散見され，整形外科医との連携も重要である．

横隔膜ヘルニアでは横隔膜全欠損や高度肺低形成などの重症症例の救命例では，反復する呼吸器感染，気管支喘息，慢性肺機能障害，慢性肺高血圧症，胃食道逆流症，逆流性食道炎，栄養障害に伴う成長障害，精神運動発達遅滞，聴力障害，漏斗胸，脊椎側弯などを発症しやすい．生存例の15～30%程度にこれらの後遺症や障害を伴うと言われ，新生児外科治療の改善による重症症例の救命例の増加に伴い，長期にわたり在宅酸素療法が必要な症例を含む後遺症や障害を有する症例が，今後一層増加すると考えられ，本症の長期フォローアップと治療の継続が重要で，総合診療医を中心とした地域連携も必要である．

2. 乳幼児期外科疾患の長期予後

胆道閉鎖症例では約1/3が自己肝で成人に達するが，小児期に肝移植を受けた患児は一生免疫抑制薬の服用を続ける必要があり，服薬のコンプライアンスの問題のみならず長期薬剤服用の副作用として，感染症，腎障害，糖尿病，高血圧，発がんなどのリスクがある．したがって，成人期以降は内科医による免疫抑制薬の継続投与と，長期的な合併症に留意した定期的健診が必要となる．

胆道拡張症では成人期になってから，吻合部狭窄，肝内結石，胆管炎，胆管がんの発症を認めることがあるため，小児外科でのフォローアップにとどまらず，成人肝胆膵外科との連携も重要である．

小児がんの治療成績の劇的な向上により，進行例でも治療後に長期生存する症例が増加してきた．こういう症例では成長障害，腎機能障害，内分泌・代謝障害だけでなく，10年後，20年後の晩期再発のみならず二次がん発症のリスクもあり，長期フォローアップを含め，成人血液・腫瘍内科医との連携も念頭に入れるべきである．

ヒルシュスプルング病類縁疾患は直腸に神経節細胞が存在するにもかかわらず，ヒルシュスプルング病のような機能性腸閉塞症を来す疾患で，このうちhypoganglionosis（神経節細胞減少症），MMIHS（megacystis-microcolon-intestinal hypoperistalsis syndrome；巨大膀胱短小結腸腸管蠕動不全症），CIIP（chronic idiopathic intestinal pseudo-obstruction；慢性特発性偽性腸閉塞症）は全消化管に異常があり，長期的な静脈栄養や経腸栄養，腸瘻の管理など，成人に至るまで治療が必要である．現在，厚生労働科学研究の研究班（研究代表者：田口智章）にて，全国実態調査を基に小児から成人に至るシームレスな診断基準，重症度分類，ガイドラ

インの整備が行われており[1]，平成27年1月から小児慢性特定疾患および難病指定として成人期まで医療費補助が開始される予定である．

3. 重症心身障害児に対する小児外科医の関与とその予後

重症心身障害児は小児科医が成人期に到達してもずっと診療しているのが現状である．新生児仮死や低酸素性脳症による脳性麻痺，重症てんかん，脳炎，髄膜炎の後遺症などは，新生児期から20歳を過ぎても重症心身障害児療育施設または在宅で，小児科医により診療や経過観察されているのが一般的のようである．それらの症例に外科的処置が必要になった場合に，小児外科医が対応しているのが現状である．

胃食道逆流症，誤嚥による嚥下性肺炎の防止などが主な外科的な使命であり，栄養路の確保・誤嚥防止目的で胃瘻造設術が，重度の胃食道逆流症には噴門形成術が，気道確保や誤嚥防止目的に気管切開術や喉頭気管分離術が行われている．また，気管切開例では気管腕頭動脈瘻による出血を認めることがあり，腕頭動脈離断術が行われることもある．そのほか，消化性潰瘍からの出血や穿孔，腫瘍の発生なども散見される．ある程度広い範囲の臓器に臨機応変に対応できるのが小児外科医の強みであり，これに代われる成人外科医は今のところ養成されていないのが現状である．

II 小児外科疾患におけるトランジションの必要性と問題点

昨今，小児期発症疾患を有する患児の成人期に向かう診療にあたって，小児期医療から個々の患者にふさわしい成人期医療への移り変わり，すなわち，移行期医療（トランジション）が重要な課題となっている．移行期においていかなる医療を受けるかの決定権は患者にあり，患者およびその家族の望まない成人診療科への転科を勧めるために使われてはならないのは言うまでもない．そのために患者本人が理解力と判断力に応じた説明を受け決定したり，意見を表明できることが重要で，成人期の小児期発症疾患に対しては，年齢と共に変化する病態の研究，適切な診療方法の開発が不可欠である．同時に，病態の変化と人格の成熟に伴い，小児期医療から成人期医療へ移行する間で，これら2つの医療の担い手が，シームレスな医療を提供することが肝要である．

小児期に手術を受けた患児のうち，手術で病気が治癒し通常の日常生活を営むことが可能で，問題なく成長し成人に達する者が多数を占めている．しかしながら，一部の患児は術後も排便・排尿障害や腎障害，運動障害などが継続し，長期的な治療が必要な例もあり，思春期・成人期に達すると性や生殖の問題も生じてくるのも現状で，トランジションが問題となってくる．

現在，小児期に手術を受け，長期にわたり治療やフォローアップが必要な患児は，思春期や青年期になっても小児外科医が窓口になって，それぞれの臓器に特異的な診療科である耳鼻科，泌尿器科，産婦人科や内科と連携しながら診療している場合が多い．しかし，成人期になると小児期にはないような病気，つまり成人病や成人特有の上皮由来のがんなどの発症があるため，成人としての健診を定期的に受診し，成人期の病気を見逃さないようにしなければならない．そのためには患者に自分は成人であると自覚させ，それから先の展望を十分に説明し，小児外科医から巣立っていく必要がある．しかしながら，患児と医師には小児期の病気を介した濃い人間関係ができていることが多く，それらの関係を継続したまま成人期まで小児外科医が診療にあたるのは時間的制約もあり，お互いにとって不利益の場合も多い．

小児期に発症し成人期にも認められる疾患である潰瘍性大腸炎やクローン病などは，むしろ内科医のほうが得意な疾患なので，小児系診療科から成人診療科へのスムーズな移行が可能と

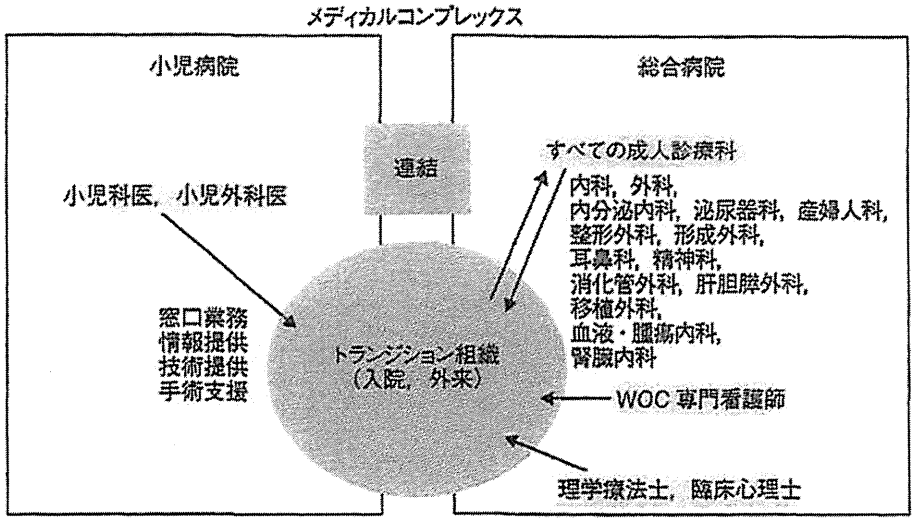

図1　トランジション組織

思われる．しかし新生児外科疾患など，通常，成人内科医や成人外科医が診療にあたることのない疾患が問題となる．実際，これらの疾患では小児外科医が成人期になっても診察していることが多く，小児期からの濃い人間関係に起因し，患児は旧知の小児外科医に頼り自立しにくいという問題が生じているのも事実である．

このような症例に対し，総合病院での主治医は内科医（総合診療医）が担当し，問題のある臓器は臓器別に耳鼻科，泌尿器科，産婦人科や成人の外科に相談するという診療体制（トランジション組織）を構築し，小児期の情報は小児科医や小児外科医が詳細に提供するという形が望ましい．小児病院ではこの形態は取れないので，総合病院との連結，すなわちメディカルコンプレックス型の病院の整備が必要となる（図1）．

おわりに

小児外科疾患は希少難病が多いため，生涯にわたるシームレスな医療の提供が必要であるが，病気について理解しているのは小児外科医のため，小児外科医が主体となりがちである．患児側も医師側も小児期の病気を介した濃い人間関係が良くも悪くも影響し，疾患の種類によっては成人診療科に引き渡す受け皿がなく，小児外科で医療を継続的に行っていかなければならない症例があるのも現状である．このような状況下でも，成人期に達した患者およびその家族に対する十分な説明と理解の下で可能な限り小児期医療から成人期医療へ移行するにあたり，成人診療科と連携しながらできるだけシームレスな医療を提供することが肝要である．

日本小児外科学会ではこの問題に関してトランジション検討委員会を立ち上げ，調査活動を開始し，今後，学会としての提言をまとめる予定である．

文　献

1）平成24年度厚生労働科学研究費補助金難治性疾患等克服研究事業「小児期からの消化器系希少難治性疾患群の包括的調査研究とシームレスなガイドライン作成」（研究代表者：田口智章）総括・分担研究報告書．平成25年3月．

PEDIATRICS INTERNATIONAL Official Journal of the Japan Pediatric Society

Brain abscess in hepatopulmonary syndrome associated with biliary atresia

Keiichi Morita, Hiroaki Fukuzawa and Kosaku Maeda
Department of Pediatric surgery, Kobe Children's Hospital, Kobe, Japan

Abstract The first-choice therapy for biliary atresia (BA) is Kasai hepatoportoenterostomy, which has been shown to greatly improve outcome. Various long-term complications, however, such as portal hypertension and hepatopulmonary syndrome (HPS), can occur in patients with native liver. A rare case of brain abscess in an 11-year-old girl with HPS associated with BA is reported. The patient underwent hepatoportoenterostomy for BA at 53 days of age, with resolution of hyperbilirubinemia. At 10 years of age, she was diagnosed with severe HPS with right-to-left shunting, and preparations for liver transplantation proceeded. Three months after the diagnosis, she had a right parietal brain abscess. Given that the brain abscess enlarged in size, surgical drainage of the brain abscess was performed. The postoperative course was uneventful, but a slight left hemiplegia remained at discharge. The presumed mechanism of abscess formation in HPS may be right-to-left bacterial transit through intrapulmonary vascular dilatations and/or arteriovenous fistulae.

Key words biliary atresia, brain abscess, children, hepatopulmonary syndrome.

The outcome for patients with biliary atresia (BA) has improved since the introduction of Kasai hepatoportoenterostomy, the first-line therapeutic procedure to relieve jaundice. Various long-term complications, however, such as portal hypertension and hepatopulmonary syndrome (HPS), have been reported in patients with native liver. A rare pediatric case of brain abscess in HPS associated with BA is reported.

Case report

An 11-year-old girl underwent hepatoportoenterostomy for type III BA at 53 days of age. Hyperbilirubinemia promptly resolved after the operation, and serum total bilirubin was <1.5 mg/dL. She had recurrent cholangitis and esophageal varices in early infancy, but both were well controlled. At 10 years of age, although she had no respiratory symptoms, oxygen desaturation was noted. Oxygen saturation on room air was 89%. On arterial blood gas analysis, partial pressure of oxygen was 50.9 mmHg and alveolar–arterial oxygen gradient was 63.2 mmHg on room air. Pulmonary angiography demonstrated early return of blood flow from the pulmonary artery to the pulmonary vein. Transthoracic contrast echocardiography was performed at the same time as pulmonary angiography. When the agitated saline with microbubbles was injected into the pulmonary artery, microbubbles appeared immediately in the left atrium, suggesting right-to-left shunting (Fig. 1). The rate of right-to-left shunting on lung scanning with ^{99m}Tc-labeled macroaggregated albumin was 35% (Fig. 2). The patient was therefore diagnosed with severe HPS associated with BA, and preparations for liver transplantation proceeded.

Three months after the diagnosis of HPS, the patient was admitted to hospital for sudden convulsions of the face and left upper limb. She was alert and conscious, and had a slight fever of 37.8°C. Blood pressure was 106/69 mmHg, and the pulse rate was 112 beats/min. Respiratory rate was 18 breaths/min, and oxygen saturation on room air was 90%. Heart and lung examinations were normal. The liver was firm at 2 cm below the right costal margin, and digital clubbing was present. Laboratory results were as follows: leukocytes, 5100/mm^3; C-reactive protein, 0.26 mg/dL; total bilirubin, 3.06 mg/dL; and no polycythemia. Brain magnetic resonance imaging demonstrated a right parietal brain abscess that appeared slightly hypointense on T1-weighted and hyperintense on T2-weighted imaging (Fig. 1). A distant infection focus, contiguous infection with the abscess, or cranial injury was not detected. Furthermore, history of infectious disease, such as otitis media, sinusitis, or dental infection, or neurological procedures was not present. On the fourth day, the convulsions could not be controlled, and brain computed tomography showed enlargement of the brain abscess and midline shift. Therefore, surgical drainage of the brain abscess was undertaken on the fifth day, and the purulent drainage material was found to contain viridans streptococci. Viridans streptococci were sensitive to ampicillin and cefazolin. The postoperative course was uneventful, and the patient received a 6 week course of i.v. antibiotics (ampicillin). The neurological symptoms improved gradually, but a slight left hemiplegia remained at discharge on the 64th day.

Discussion

Biliary atresia is a rare disease that causes obliterative cholangiopathy that affects varying lengths of both intrahepatic and extrahepatic bile ducts. Kasai hepatoportoenterostomy is widely

Correspondence: Keiichi Morita, MD, Department of Pediatric Surgery, Kobe Children's Hospital, 1-1-1 Takakuradai, Suma-ku, Kobe 654–0081, Japan. Email: morita_kch@hp.pref.hyogo.jp

Received 8 February 2015; revised 11 April 2015; accepted 21 April 2015.
doi: 10.1111/ped.12711

日小外会誌　第51巻1号　2015年2月，pp. 80-86
doi: 10.11164/jjsps.51.1_80

先天性胆道拡張症術後の胆管および膵管に関連する合併症

福澤　宏明，武本　淳吉，玉城　昭彦，森田　圭一，岩出　珠幾，
大片　祐一，遠藤　耕介，尾藤　祐子，横井　暁子，前田　貢作

要　旨

先天性胆道拡張症に対する根治術により多くの症例は問題なく経過するが，胆管・膵管に絡む合併症の報告も散見される．胆管系・膵管系合併症例を初回手術時の膵・胆管合流形態を含め後方視的に検討した．1995年4月から2013年12月に当院で先天性胆道拡張症手術を施行されたのは105例あり，その中で胆管系合併症を5例，膵管系合併症を3例に認めた．胆管系合併症5例のうち3例に肝内結石を認め，これらの症例は左右肝管起始部に相対的な狭窄を認めていた．膵管系合併症の2例に拡張した共通管内に蛋白栓を認めた．これら2例の膵・胆管合流形態は，拡張した共通管に非常に細い下部胆管が直角に合流する形態（新古味分類Ib）であった．膵管系の合併症のもう1例は，不完全型膵管癒合不全を合併しており副乳頭切開が施行された．非常に拡張した共通管を持つ症例の中に，術後共通管内に蛋白栓を認め膵炎を発症するものがあり注意が必要である．

索引用語：先天性胆道拡張症，膵・胆管合流異常症，結石，膵炎，合併症

I　はじめに

現在，小児の先天性胆道拡張症に対する根治術（分流手術）は，ほぼ確立された術式となっており，この分流手術により多くの症例は問題なく経過する．しかし中には経過中に胆管系または膵管系の合併症を起こす例が散見される．これらの合併症として，吻合部狭窄，胆管炎，肝内結石，膵石，膵炎，発癌などが挙げられているが，大きく胆管系の合併症と膵管系の合併症に分けて考えることができる．胆管系合併症の代表である肝内結石は，戸谷IV-Aのような左右肝管起始部に相対的な狭窄の存在する症例に高率に起こると言われている．しかし，先天性胆道拡張症後の膵炎に関しては，どのような膵・胆管合流形態に発生しやすいかという報告は少ない[1)2)]．

今回，当院で根治手術を施行した先天性胆道拡張症をもとに，術後の胆管系・膵管系合併症例の初回手術時の胆管・膵管の形態を調べ，その特徴を検討した．

II　症　　例

1995年4月から2013年12月までに当院で根治術が施行された先天性胆道拡張症は105例であった．その内，術後に胆管系・膵管系に合併症を認めたのは8例（7.6%）存在した．5例（4.8%）は胆管炎，肝内結石などの胆管系合併症，3例（2.8%）は膵管系合併症である膵炎を発症した．

胆管系合併症を表1に示す．胆管系合併症のうち，肝内結石を3例（症例1，2，3）に認め，結石を伴わない胆管炎を2例（症例4，5）に認めた．肝内結石を認めた3例の初回手術時の胆管形態は，いずれも戸谷IV-Aで肝内胆管の拡張を伴っていた．肝内結石の発症時期は術後9年，16年，7年といずれも根治術からかなり経てから発症していた．また肝内結石の発生部位は，初回手術時に肝内胆管に拡張を認めていた部位であった．肝内結石を形成せず胆管炎のみを発症した2例は，両葉にわたる肝内胆管の軽度拡張を認めた．

胆管系合併症を提示する．なお胆管の形態は戸谷分類[3)]で示した．

【症例1】左肝管起始部に相対的狭窄を伴う戸谷IV-A型の先天性胆道拡張症に対し，相対的狭窄を残して拡張胆管空腸吻合術が施行された（図1a）．根治術後9年目に胆管炎を発症，CTにて肝左葉の肝内胆管拡張を認め，肝内結石を認めた（図1b）．絶食にて結石は，1週間で自然排出し，その後5年間症状なく経過している．

【症例2】右肝管起始部が嚢胞状に拡張している戸谷IV-Aに対して，右肝管に切り上げて，相対的狭窄を開

兵庫県立こども病院小児外科
代表者連絡先：福澤宏明　〒654-0081 神戸市須磨区高倉台1-1-1
兵庫県立こども病院小児外科

表1　胆管系合併症

症例	根治術時年齢（歳）	合併症	トラブルまでの期間	胆管の形態	胆管空腸吻合位置	転帰
1	2.49	肝内結石	9年	IV-A（左右肝管起始部に相対狭窄）	総肝管で吻合	自然に結石は排出されたその後5年症状なし
2	1.86	肝内結石	16年	IV-A（右肝管が嚢胞状に拡張）	右肝管の拡張部に切り込んで吻合	他院にて治療中
3	1.81	肝内結石	7年	IV-A（左肝管起始部に相対狭窄）	左肝管に切り込んで吻合	自然に結石は排出されたその後12年症状なし
4	2.13	繰り返す胆管炎	1年	Ic	総肝管で吻合	現在も胆管炎を繰り返す
5	0.39	胆管炎	11年	Ia	総肝管で吻合	現在は症状なしその後1年症状なし

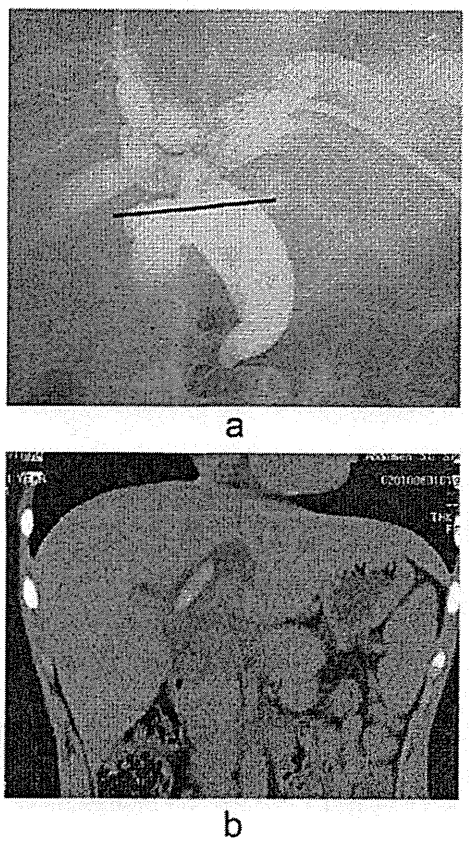

図1　a　症例1：戸谷IV-A型，総肝管レベルで吻合（実線）．b　症例1：左肝管内に結石

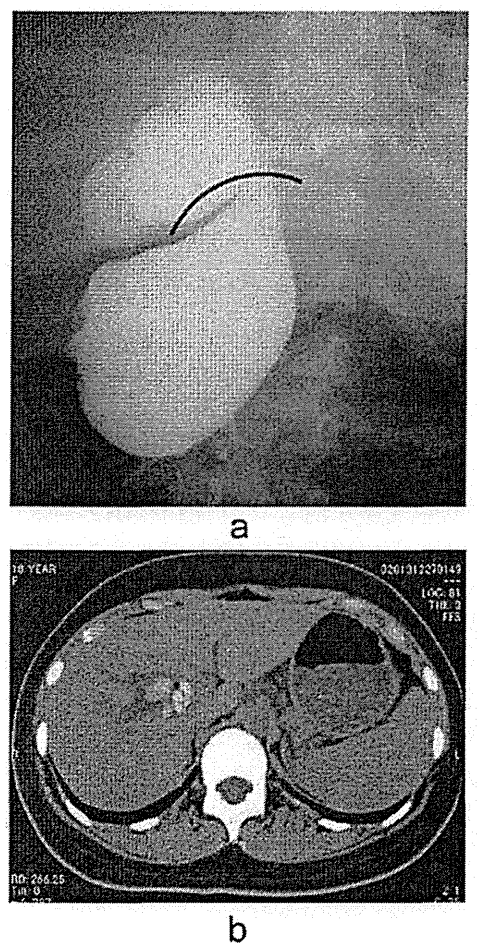

図2　a　症例2：右肝内胆管が嚢胞状に拡張．拡張した胆管を開放し，肝管空腸吻合が施行された（実線）．b　症例2：根治術後16年目に右肝内胆管に結石を認めた．

放するように肝管空腸吻合が施行された（図2a）．しかし，根治術後16年目に嚢胞状に拡張した右肝管に結石を認めた（図2b）．現在他院で治療中である．

【症例3】左肝管起始部に相対的狭窄を認める戸谷IV-A症例に対し，相対的狭窄を解除すべく左肝管に切り上げて肝管空腸吻合が施行された（図3a）．根治術後7年目に胆管炎で発症し，CTにて肝左葉の肝内胆管拡張と同部に結石を認めた（図3b）．これに対し，ウルソデオキシコール酸を内服したところ，結石は4日目に自然排出された．その後12年症状なく経過している．

【症例4】戸谷Icの先天性胆道拡張症に対し，総肝管での肝管空腸吻合が施行された（Roux-en-Y脚は40 cm）．

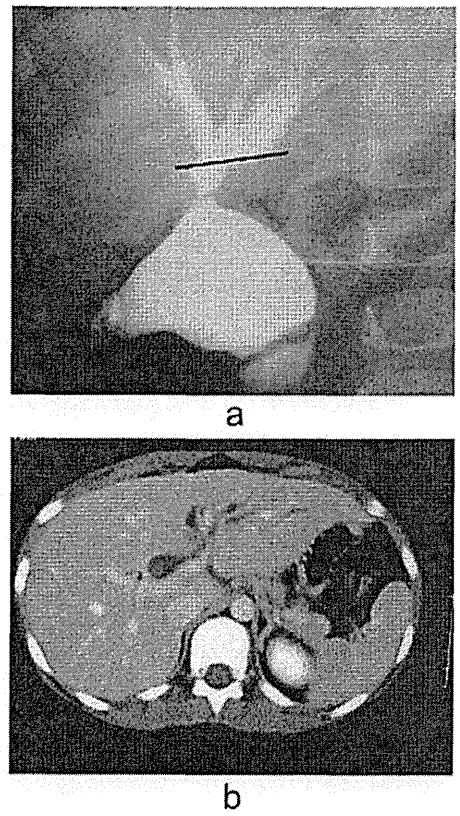

図3 a 症例3：左肝管起始部に相対的狭窄を認め，左肝管に切り上げて吻合（実線）．b 症例3：左肝管に結石影を認め，肝左葉の肝内胆管拡張が見られる．

根治術後1年目より，月1回程度の胆管炎を繰り返している．CTで両葉の軽度胆管拡張と中等度の胆管気腫を認めるが，肝内結石は認めない．DIC-CTでは肝内胆管拡張は軽度であるが，長く蛇行するRoux-en-Y脚を認めた．Roux-en-Y脚の輸送能に問題があると考えており，再手術（Roux-en-Y脚を短くし，胆管空腸の再吻合）を予定している．

【症例5】新生児期発症のIa型に対し，総肝管空腸吻合が施行された．根治術後11年目に，両葉にわたる肝内胆管拡張を伴う胆管炎を発症．保存的に軽快した．その後1年症状なく経過している．

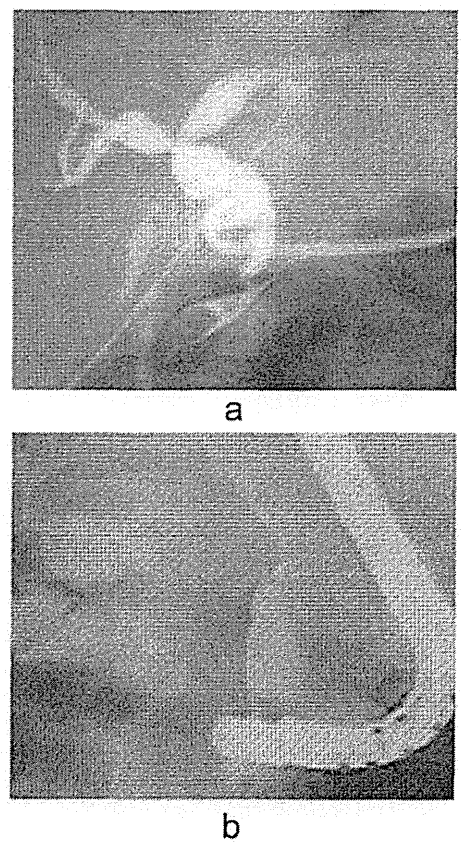

図4 a 症例6：術前Tチューブからの胆道造影，新古味分類Ibの合流形態で共通管内に陰影欠損を認める．b 症例6：膵炎後のERCP．拡張した共通管は依然存在していた．また陰影欠損，膵内の遺残胆管は見られなかった．

膵管系合併症を表2に示す．膵管系合併症（膵炎）は3例に認めた．2例は拡張した共通管内の蛋白栓が原因と考えられ，1例は不完全型膵管癒合不全が原因と考えられた．

膵管系合併症を提示する．なお膵・胆管合流形態は新古味分類[1]で示した．

【症例6】胆道穿孔で発症した先天性胆道拡張症に対し，胆管Tチューブドレナージが施行された．合流形態は新古味分類Ibで，非常に細くなった下部胆管が拡

表2 膵管系合併症

症例	根治術時年齢（歳）	合併症	トラブルまでの期間	胆管の形態	膵・胆管の合流形態	処置	転帰
6	1.25	膵炎	2年0か月	IV-A	Ib（共通管に蛋白栓）	内視鏡下主乳頭切開	症状軽快 その後6年症状なし
7	4.47	膵炎	21日	IV-A	Ib（共通管に蛋白栓）	ERCPのみ	症状軽快 その後16年症状なし
8	8.46	膵炎	1年2か月	非拡張	III-C2	内視鏡下副乳頭切開	膵炎残存

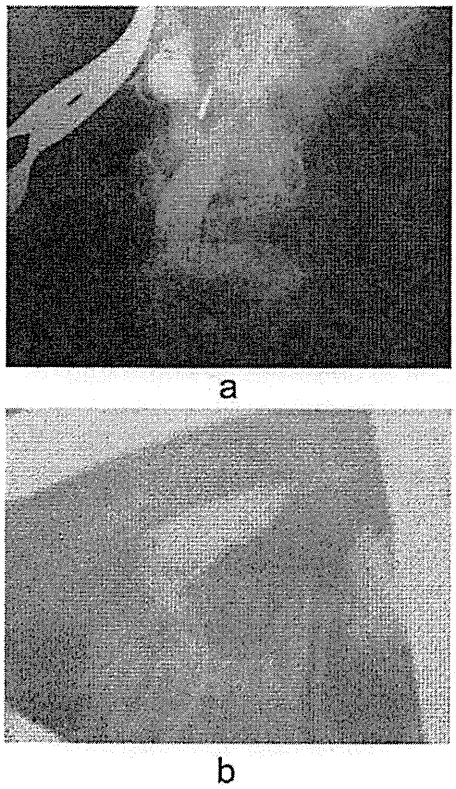

図5　a　症例7：術中胆道造影で新古味分類Ibの合流形態を，拡張した共通管内に陰影欠損を認めた．b　症例7：術後ERCP．拡張した共通管は認めるが，陰影欠損，膵内遺残胆管は見られなかった．

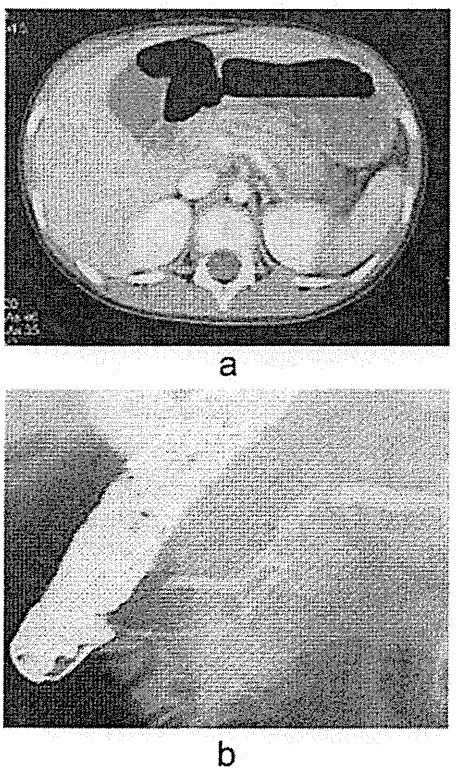

図6　a　症例8：発症時のCT．膵腫大と膵周囲の浮腫を認め膵炎と診断．b　症例8：術後ERCP（主乳頭より）．細く蛇行したWirsung管と太いSantorini管を認めることより，不完全型の膵管癒合不全と考えられる．

張した共通管膵管に直角に合流していた（図4a）．術中造影で拡張した共通管には蛋白栓と思われる陰影欠損を認めた．術後2年目に膵管拡張を伴う膵炎を発症．エコー検査にて拡張した共通管内に蛋白栓と思われる高エコー域を認めた．膵炎は保存的に軽快し，その後施行したERCPで，拡張した共通管は依然存在していたが，下部胆管の遺残は見られなかった（図4b）．乳頭機能に問題があると考えられ，内視鏡下主乳頭切開が施行され，その後6年間無症状で経過している．

【症例7】膵・胆管合流形態が新古味分類Ibの先天性胆道拡張症に対し根治術施行．術中胆道造影で共通管に陰影欠損を認めた（図5a）．術後早期から膵炎を発症，エコー検査にて拡張した共通管内に蛋白栓と思われる高エコー域を認めた．根治術後21日目にERCPが施行された．拡張した共通管は依然存在していたが，胆管の遺残は認めなかった（図5b）．ERCP施行時は拡張した共通管内に陰影欠損を認めず，乳頭切開は行われなかった．その後16年症状なく経過している．

【症例8】高アミラーゼ血症を伴う腹痛で発症し，CT検査で膵管拡張を伴う膵腫大と膵周囲の浮腫を認める膵炎と診断（図6a）．ERCPにて胆管非拡張の膵・胆管合流異常をみとめ，これに対し根治手術が施行された．しかし術後1年目より膵炎を繰り返すようになった．ERCPでは，細く屈曲した腹側膵管と太い背側膵管を認め，不完全型膵管癒合不全の存在が疑われた（図6b）．不完全型膵管癒合不全が膵炎の原因と考えられ，内視鏡下副乳頭切開が施行されたが現在も膵炎を繰り返し治療に難渋している．

III　考　　察

膵・胆管合流異常は，解剖学的に膵管と胆管が十二指腸壁外で合流する先天奇形と定義され，先天性胆道拡張症のほぼ全例に認められる．機能的に十二指腸乳頭部括約筋（Oddi筋）の作用が合流部に及ばないため，膵液と胆汁の相互逆流が起こり，胆道ないし膵に種々の病態を引き起こし得る病態である[4]．小児では胆管拡張のあるものはもちろん，胆管非拡張症例においても将来の発癌を懸念して分流手術がなされることが一般的となっている[5]．多くの症例は分流手術後問題なく経過するが，中に胆管系あるいは膵管系の合併症を認める報告があり

問題となっている[6)-9)].

胆管系の合併症の代表として肝内結石が挙げられる．安藤ら[10]はIV-A型の術後に2～13年の観察期間で32%に肝内結石が生じたと報告しており，これは胆道拡張症全体の術後肝内結石の発生率（7～8%）[11)12)]と比べると高い．IV-A型は肝門部または肝内胆管に相対的な胆管狭窄が存在し，これら相対的狭窄を残存した形で再建すると，術後の肝内結石の原因となると言われており[13)]，IV-A型は術後肝内結石の危険因子であると考えられている．また，先天性胆道拡張症の肝内胆管には7～8割の例に狭窄が見られるとの報告もある．これら狭窄には膜様・索状などの形態があり，狭窄のほとんどが肝門部付近に存在している[14)]．術後結石形成予防のために，これらの狭窄は手術時に見落とすことなく対処する必要があるとされている．

当院で経験した肝内結石3例はいずれも戸谷IV-A型であり，初回手術時に左右肝管起始部に相対的狭窄（高度狭窄を認める症例はなかった）を認めていた．また，発症時期は初回手術から7年，9年，16年とかなり経っていた．肝内結石の発症時期に関しては，術後5か月から20年であったとの報告もあり[15)]，術後肝内結石の形成には比較的長い年月がかかることが推測された．

我々の経験した肝内結石3例はいずれも，左右どちらかの肝内胆管に発生していた．結石形成をきたした肝内胆管は，初回手術時にはすでに拡張を認め，肝管起始部に相対的狭窄も認めていた．1例は初回手術時に狭窄を残したまま肝管空腸吻合がなされており，2例は狭窄を解除するように切り上げて吻合がなされていた．その2例は胆管を左右に切り上げて吻合されていたにもかかわらず，拡張した肝内胆管に結石を形成した．

その理由として狭窄の解除が不十分であった，もしくは切り上げた胆管の再癒合などの理由で，拡張した肝内胆管内の胆汁のドレナージ不良が生じたと推測される．初回手術時に肝門部胆管に相対的狭窄があり肝内胆管拡張がある症例では，肝門部胆管の胆管形成を行っても，肝内結石を形成する症例があった．幸いなことに，肝内結石を形成した3例中2例は保存的に結石が排出されたが，結石の原因が肝内胆管の胆汁の流出障害であるとすると，いずれまた肝内結石の再形成を来すことが予想される．肝内結石に対するアプローチに関しては非観血的治療もあるが，仮に結石を除去できても相対的狭窄による肝内胆汁ドレナージ不良が残存し，再度肝内結石を形成してくるため，肝切除を行うべきとの意見もある[16)]．肝切除まで行うかどうかについては議論のあるところであるが，再度肝内結石を形成したときは，少なくとも肝門部胆管の形成を加えた肝門部肝管空腸再吻合を行うべきであると考えている．

肝内結石を伴わない胆管炎は2例に認めた．1例（症例5）は術後11年目に軽度肝内胆管拡張を伴うもので保存的に軽快した．肝内胆管拡張は両葉にわたり存在し，おそらく吻合部の軽度狭窄に起因したものであると推測される．もう1例（症例4）は術後1年目より繰り返す胆管炎で，肝内結石は認めず中等度の胆管気腫を認める．DIC-CTにてRoux-en-Y脚が非常に長く蛇行しており，造影剤の通過が悪い所見が得られた．こちらは，過長な屈曲したRoux-en-Y脚により胆汁の輸送能に問題があると考えられ現在再手術待機中である．現在当院では根治手術の際，Roux-en-Y脚の横行結腸間膜より頭側の部分をなるべく短くすることにより，肝門部付近での蛇行が少なくなるように心がけている．

先天性胆道拡張症術後の膵炎は合併症の1つとして挙げられるが，胆管系の合併症に比してその頻度は少ない．分流手術後の膵炎の原因の1つに，根治術の際の下部胆管の遺残が挙げられる．膵頭部に遺残する拡張胆管とその内部への膵液の逆流とうっ滞により膵液の排出が障害され膵炎を惹起することが原因と考えられている．Koshinagaら[8)]は，根治術後に遺残した下部胆管内に膵石を形成した3例を報告している．それによると根治術後6年，15年，15年といずれも長期経過後に発症しており，別の報告[15)]でも分流手術後5年，6年，27年後に発症した遺残胆管内の結石形成が報告されている．そのことから下部胆管の遺残が原因での膵炎は術後長期経過し発症してくることが予想される．

またもう1つの術後膵炎の原因として，膵頭部膵管の形態異常による膵液の排出障害が挙げられる．吾妻ら[9)]は，術後早期に膵炎を発症した遺残胆管のない症例を報告し，拡張した膵管内に膵石を認めたことから，術後膵炎の発症には膵頭部における膵管の形態異常が重要であると述べている．我々が経験した，術後膵炎2例（症例6，7）は，下部胆管が非常に細く，共通管内の蛋白栓に術中アプローチする事が比較的難しい合流形態と考えられる．膵炎発症時のERCPで遺残胆管はなく，非常に拡張した共通管（膵管）を認め，手術時の造影でどちらも共通管内に陰影欠損を認めていたことから，拡張した膵管内の物質（おそらく蛋白栓）による膵液の排出障害が原因と推測される．この蛋白栓が，分流手術時から存在していたものか分流手術後に形成されたものかは議論になるところである．

Kanekoらによると，膵・胆管合流異常症における蛋白栓はほとんどが，Lithostathineであり，トリプシノー