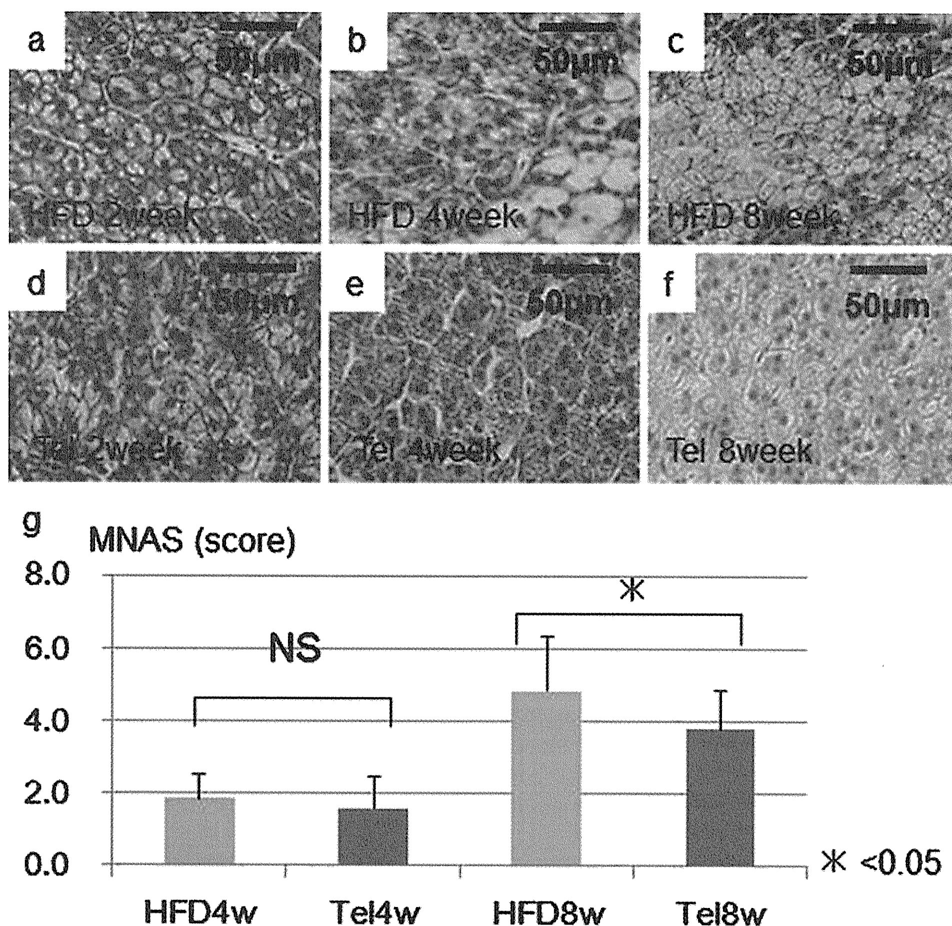

Fig. 4 Medaka histology demonstrated lipid droplet deposition in hepatocytes after 2 weeks of HFD (**a**). Lipid deposition in hepatocytes was striking and clear cell formation was observed after 4 weeks (**b**). Degenerated hepatocytes were seen at 8 weeks (**c**). In the Tel group (1 ppm), hepatic steatosis was attenuated over the same period (**d–f**). In terms of MNAS, a significant difference was seen between the Tel group (1.6±0.9 after 4 weeks [*Tel4w*], 3.8±1.0 after 8 weeks [*Tel8w*], $P<0.05$) and the HFD group (1.9±0.7 after 4 weeks [*HFD4w*], 4.9±1.5 after 8 weeks [*HFD8w*], $P<0.05$) with the Tel group showing improved MNAS (Tel group after 8 weeks at 3.8±1.0 vs HFD group after 8 weeks at 4.9±1.5; $*P<0.05$; *NS* nonsignificant; **g**)

administration in the present study does not seem to ameliorate the n3/n6 PUFA ratio imbalance in the liver (Table 4). Ballooning degeneration is assumed to be related to changes in the lipid composition of the fatty acid bilayer in the cellular membrane and to the n3/n6 PUFA ratio. Although no change in the n3/n6 PUFA ratio of the liver fatty acid fraction attributable to Tel administration has been found, Tel ameliorates ballooning degeneration.

The medaka NASH model shows increased liver tissue infiltration by D-PAS-positive and CD68-positive macrophages over time from the start of HFD (Figs. 2, 5b-c) but such cellular infiltrations are suppressed by Tel administration. These results suggest that the ballooning degeneration of hepatocytes might be closely regulated by macrophage infiltration. More detailed investigations of the relationship between ballooning degeneration and changes in the lipid composition of the fatty acid bilayer in the cellular membrane are needed.

These observations indicate that progression of NASH pathology is linked to the infiltration of macrophages into liver tissue and that Tel is effective as a dietary treatment to suppress such infiltration. A recent study has suggested that an increase in the number of macrophages infiltrating adipose tissue is correlated with obesity (Weisberg et al. 2003). Activated monocytes have been found in the arterial walls of humans and other animals with hypertension (Ishibashi et

Table 3 Triglyceride (mg/dl) changes in medaka blood (1 sample=10 fish; *HFD* high-fat diet, *Tel* telmisartan, *w* week)

Treatment	Total	Chylomicron	Very low-density lipoprotein	Low-density lipoprotein	High-density lipoprotein
Normal	407.4	6.0	107.9	38.4	255.1
HFD4w	955.9	179.6	353.0	65.0	358.2
HFD8w	1352.9	303.4	571.8	107.3	370.5
Tel4w	1035.1	188.1	403.2	91.3	352.6
Tel8w	1490.8	267.3	649.0	133.8	440.7

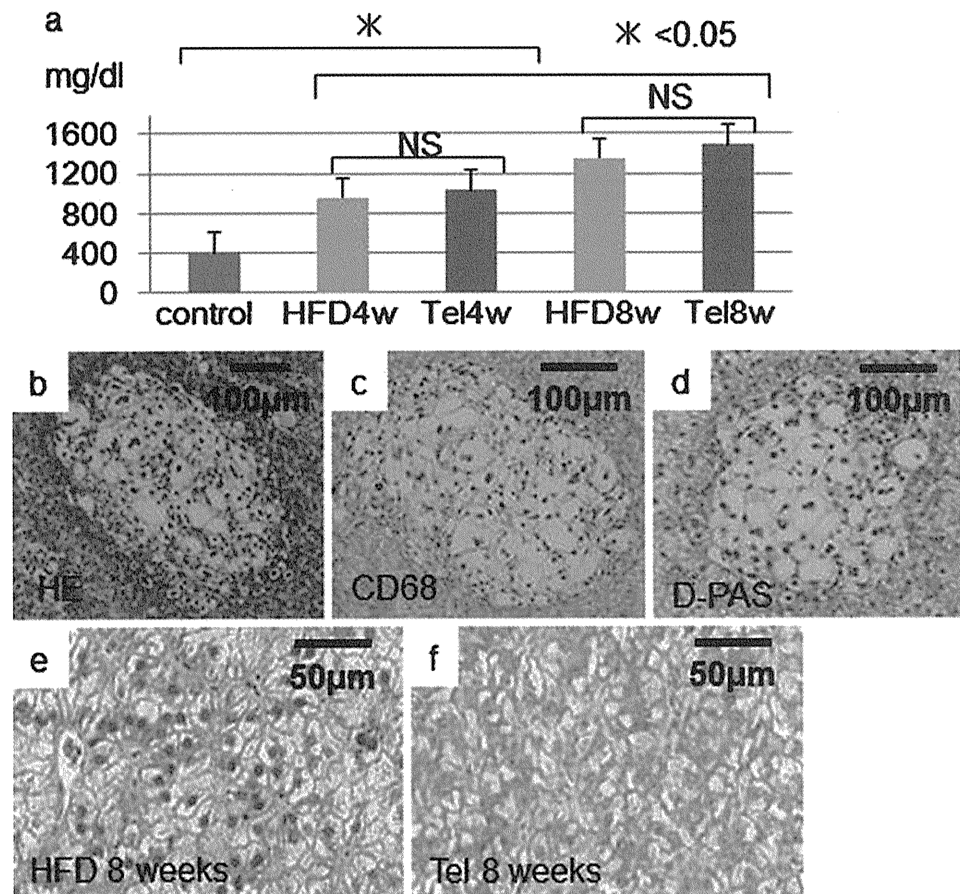

Fig. 5 Blood tests for TG were carried out on the control, HFD and Tel groups at 4 and 8 weeks (**a**). Compared with the control group, TG increased over time in the HFD and Tel groups. The TG level of the Tel group was slightly higher than that of HFD group. We observed tissue with locally aggregated ballooning hepatocyte degeneration in some areas of medaka NASH model liver tissue (**b**). Cells positive for diastase-periodic-acid-Schiff (*D-PAS*) were found at each location (**d**) and cells positive for the macrophage immunostain CD68 were also found (**c**). The HFD group showed many more cells with nuclei positive for 8-hydroxydeoxyguanosine (**e**) compared with the Tel group (**f**)

al. 2004; Weisberg et al. 2006). Moreover, insulin resistance and arteriosclerosis are clearly suppressed in CC chemokine receptor 2 (CCR2)-deficient mice (Ishibashi et al. 2004; Weisberg et al. 2006). Haukeland et al. (2006) report that serum concentrations of monocyte chemotactic protein 1 (MCP1) begin to increase with simple steatosis, peaking with NASH. These findings imply that macrophage infiltration plays an important role in the initiation of metabolic syndrome. MCP1 induces the migration of mononuclear leukocytes by combining with complement receptor type 2 (Fox et al. 1997). A recent study has shown that PPAR-γ agonists inhibit CCR2 expression in mononuclear leukocytes (Tanaka et al. 2005). Tel has also been reported to suppress macrophage infiltration by blocking the MCP1/CCR2 pathway in a mouse NASH model (Kudo et al. 2009). In our medaka NASH model, Tel might suppress macrophage migration to the liver.

In this medaka NASH model, oxidative stress has been evaluated by 8-OHdG immunostaining and has been found to be attenuated by Tel administration (Fig. 5e, f). Hepatocyte apoptosis has been reported as being the key to the second "hit" in the progression from simple steatosis to NASH in human NAFLD and is a significant pathological characteristic of NASH (Feldstein et al. 2003). Hepatocyte apoptosis in liver tissue from the medaka NASH model increases as a result of HFD administration, suggesting that apoptosis is linked to the progression from simple steatosis to NASH and disease development in the medaka NASH model (Fig. 6). One possible underlying mechanism is that the increased apoptosis is related to oxidative stress caused by HFD administration. In the present study, the suppression of apoptosis is greater in the Tel group than in the HFD group.

With regard to liver lipid metabolism, lipid deposited in the fatty liver is in the form of TG. This is attributable to changes in the balance between TG uptake into the liver and secretion out of the liver and to the balance between intrahepatic TG synthesis and breakdown. In our medaka NASH model, the influx of fats into the liver is likely to increase as a result of HFD; greater intrahepatic TG deposition is observed in the HFD group compared with the control group. Whereas no clear difference in FAS is apparent, the reduced expression of ACC1 in the HFD group probably indicates the decrease of de novo fatty acid synthesis pathways even within the relatively short time-scale of 2–4 weeks. With respect to fatty acid oxidation pathways (CPT1 and ACO1), HFD administration appears to increase the oxidation of fatty acids, which is further

Table 4 Fatty acid composition of total lipids in livers of control, HFD medaka and HFD+Tel medaka (1 sample=10 fish; *SFA* saturated fatty acid, *MUFA* mono-unsaturated fatty acid, *PUFA* polyunsaturated fatty acid)

Fatty acid (mol%)	Control	HFD	HFD+Tel
C14 : 0	3.0	16.2	14.3
C16 : 0	48.6	179.4	202.4
C18 : 0	25.8	53.9	55.2
C16 : 1 n-7	3.9	41.7	50.8
C18 : 1 n-9	25.1	514.1	533.9
C20 : 3 n-9	0.7	17.1	16.7
C18 : 2 n-6	11.8	45.0	52.2
C18 : 3 n-6	1.4	28.2	35.3
C20 : 3 n-6	2.3	5.5	3.8
C20 : 4 n-6	11.0	31.9	32.9
C18 : 3 n-3	1.1	1.7	5.2
C20 : 5 n-3	1.8	0.0	1.1
C22 : 6 n-3	62.1	28.8	28.1
Total SFA	78.4	252.5	275.2
Total MUFA	31.3	565.1	591.8
Total PUFA	99.9	164.0	180.5
n-3 PUFA	70.4	32.0	36.1
n-6 PUFA	28.9	114.9	127.7
n-3/n-6 ratio	2.4	0.3	0.3
Total lipid (mg/g tissue)	209.6	981.6	1047.5

increased by Tel administration. We have not assessed any direct changes of TG or lipid content of the liver in this series of experiments, despite TG deposition being the primary factor for the formation of fatty liver. However, Tel ameliorates steatosis in the liver. We have also shown that CPT1 and ACO1 expression is increased in the liver on administration of Tel. Thus, the administration of Tel may induce the β-oxidation of free fatty acids in the liver of medaka and thereby also contribute to the reduction of TG in the liver. We consider that this evidence demonstrates the effectiveness of Tel on TG or the oxidation of lipids.

In this study, we have investigated the pharmaceutical effectiveness of Tel, including its potential mechanisms of action. Tel seems to improve NASH, with the inhibition of macrophage infiltration and oxidative stress in the liver, but does not affect the n3/n6 PUFA ratio in NASH liver. These results show that Tel has a different drug effect on NASH and the n3/n6 PUFA ratio. Thus, the medaka NASH model is likely to become a significant experimental model for use in future NAFLD/NASH studies.

In conclusion, we have demonstrated that the medaka model produced on HFD exhibits the same changes as those seen in human NASH pathology. Furthermore, our study confirms that Tel has an effect on this model.

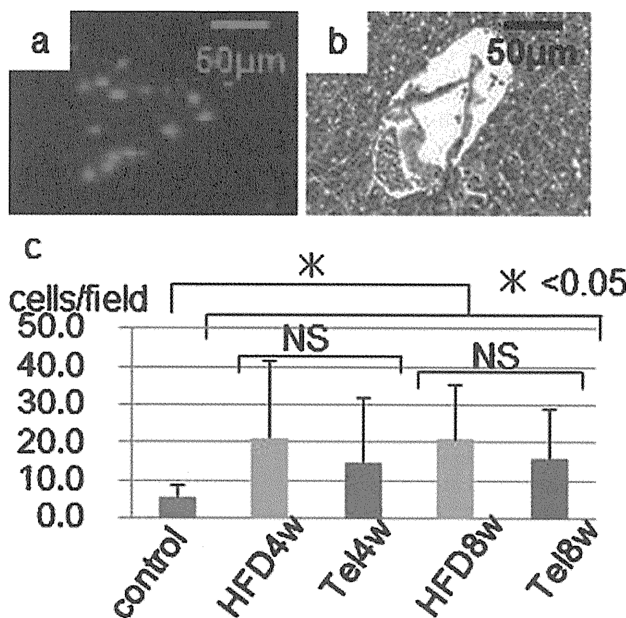

Fig. 6 **a** Investigation of induction of apoptosis-positive cells in liver tissue by TUNEL staining. **b** Same tissue with HE staining. **c** The number of apoptotic cells was greater in the HFD group at 8 weeks than in the control group at 4 weeks (**P*<0.05)

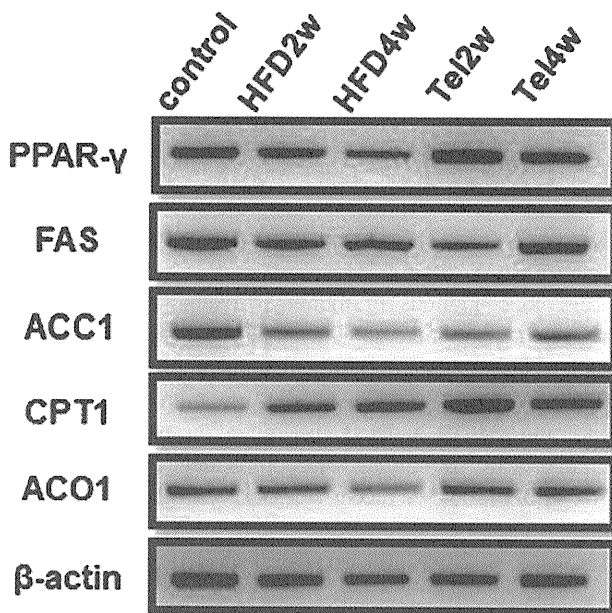

Fig. 7 PPAR-γ expression increased in the Tel group. No clear changes in expression levels of FAS were found in either the HFD or Tel groups compared with the control group. ACC1 mRNA expression in both the HFD and Tel groups was lower than in the control group. CPT1 mRNA expression was higher in the HFD group than in the control group and even higher in the Tel group. ACO1 expression was lower in the HFD group than in the control group. The expression levels of ACO1 were similar in the Tel and control groups (*PPAR-γ* peroxisome proliferator-activated receptor-γ, *FAS* fatty acid synthase, *ACC1* acetyl-CoA carboxylase 1, *CPT1* carnitine palmitoyltransferase 1, *ACO1* acyl CoA oxidase 1)

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REVIEW

Stem cell therapy in chronic liver disease

Taro Takami[a], *Shuji Terai*[b], *and Isao Sakaida*[b]

Purpose of review
To provide an overview of the current status of liver regeneration therapies for liver cirrhosis and future prospects.

Recent findings
Various clinical studies for liver disease have been reported, including hepatic administration of autologous CD34-positive cells induced by granulocyte colony-stimulating factor, portal vein administration of CD133-positive mononuclear cells, and administration of autologous bone marrow-derived mesenchymal stem cells. Effectiveness of these approaches has been shown in some patients. We have also reported improved liver fibrosis and function with infusion of autologous bone marrow cells in a basic study with mice, and on the basis of those results started autologous bone marrow cell infusion (ABM*i*) therapy for liver cirrhosis. The efficacy and safety of ABM*i* therapy has also been reported by other institutions.

Summary
Results of recent clinical studies strongly suggest that liver function-improving effects can be achieved using infusion of bone marrow (stem) cells for cirrhosis. New treatment methods using less-invasive bone marrow-derived cultured cells need to be developed.

Keywords
bone marrow, liver cirrhosis, liver regeneration, stem cell

INTRODUCTION

With the development of new antiviral medicines and advances in interferon therapy, viral elimination and cure of hepatitis can be expected even in some patients with chronic viral hepatitis, for which radical cure has been difficult. In many patients, however, liver cirrhosis has already occurred. In cases that have progressed to decompensated liver cirrhosis, the only radical treatment currently available is still liver transplantation. However, liver transplants are not widely performed worldwide due to problems such as chronic donor shortages, surgical invasiveness, risk of immunological rejection, and medical costs. To compensate for this, development of new regenerative therapies for liver cirrhosis is an urgent task. In 2000, Theise *et al.* [1] reported the existence of Y chromosome-positive cells in livers with chronic inflammation in autopsied women who had received therapeutic bone marrow transplantations from male donors, suggesting the existence of pluripotent stem cells among bone marrow cells. Since then, attention has been focused on bone marrow (stem) cells as a cell source for liver regenerative therapies [2–6,7▪▪]. Here, we present an overview of the current status of clinical trials and future prospects for liver regeneration therapies using stem cells, including autologous bone marrow-derived cells.

GRANULOCYTE COLONY-STIMULATING FACTOR

Various cell therapies for liver disease have been developed, including using CD34-positive cells induced with granulocyte colony-stimulating factor (G-CSF). Gordon *et al.* [8] collected CD34-positive cells from peripheral blood after induction with G-CSF, then administered these cells via a hepatic artery, and reported improved serum levels of bilirubin and albumin in some patients, despite a short

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KEY POINTS

- Previous clinical studies using autologous bone marrow cells suggest the efficacy and safety of this approach for advanced liver disease.
- Randomized controlled studies are needed to establish genuine efficacy because most of the published results have come from uncontrolled studies.
- New treatment methods using less-invasive bone marrow-derived cultured cell fractions showing treatment effects on liver fibrosis and functions need to be developed.

observation period of only 60 days. In addition, Spahr *et al.* [9] administered G-CSF to patients with alcoholic liver cirrhosis and reported increased proliferation of hepatic progenitor cells (HPCs), whereas Pai *et al.* [10] reported improvements in serum albumin, Child–Pugh score, and accumulation of ascites with in-vitro expansion of CD34-positive cells induced by G-CSF and administration via a hepatic artery in patients with alcoholic liver cirrhosis. A study by Han *et al.* [11] described G-CSF administration for hepatitis B virus (HBV)-related decompensated liver cirrhosis. In this study, administration of peripheral blood mononuclear cells induced with G-CSF showed significantly improved effects on serum albumin and Child–Pugh score at 6 months after treatment, compared with those in patients with only G-CSF mobilization for 4 days. However, rupture of the spleen during peripheral blood stem-cell mobilization by administration of G-CSF has been reported even in healthy individuals [12], and caution is needed in the administration of G-CSF to liver cirrhosis patients with splenomegaly.

NONCULTURED AUTOLOGOUS BONE MARROW CELLS

In our previous animal studies, we have developed an in-vivo mouse model [the green fluorescent protein (GFP)/carbon tetrachloride (CCl_4) model], and reported that GFP-positive bone marrow cells infused via a tail vein efficiently repopulated cirrhotic liver under conditions of persistent liver damage induced by CCl_4 [13]. In these processes, elevation in serum albumin levels [13], a significant increase in survival rate, and reduced liver fibrosis assessed by Sirius red staining were seen following infusion of GFP-positive bone marrow cells [14]. Infused bone marrow-derived GFP-positive cells were also confirmed to produce collagenases including matrix metalloproteinase (MMP) 2 and MMP-9 during these processes [14]. On the basis of the above basic study, infusion of autologous bone marrow cells via a peripheral vein in a chronic liver injury environment is thought to improve liver functions and reduce liver fibrosis, and to significantly improve vital prognosis in recipients. Our clinical study of autologous bone marrow cell infusion (ABM*i*) therapy for decompensated liver cirrhosis was therefore started in November 2003 [15]. In addition, a multicenter clinical trial of liver regeneration with cell transplantation was started in 2005. With that ABM*i* therapy, around 400 ml of autologous bone marrow cells was collected under general anesthesia, and the collected bone marrow fluid was concentrated and washed. Bone marrow mononuclear cells in that fluid were then purified and condensed according to standard operating procedures (SOP) at the regenerative and cell therapy center fully equipped with good manufacturing practice (GMP) grade facilities, and administered by drip infusion via a peripheral vein to the same patient. The course was observed for 6 months after ABM*i*, and efficacy and safety were evaluated using blood biochemistry tests, liver biopsy, abdominal ultrasonography, and abdominal computed tomography and so on. During the observation period, no changes in oral medications, antiviral drugs, or other agents were seen [15]. As a result, serum albumin levels, total protein levels, and Child–Pugh score at 6 months after ABM*i* were significantly improved in patients for whom the course could be observed for 6 months after ABM*i* [15]. Similar improvements were also seen in nine patients for whom the course could be observed for 15 months [16]. In 2011, Kim *et al.* [17▪] reported that ABM*i* improved serum albumin levels, Child–Pugh score, liver volume measured by MRI and accumulation of ascites in patients with HBV-related decompensated liver cirrhosis, and results from liver biopsies taken over time suggested the possibility of HPC activation as the underlying mechanism. All 10 patients who received ABM*i* also showed an improvement in quality of life, with no serious adverse events. Moreover, Saito *et al.* [18▪] recently reported the efficacy and safety of ABM*i* therapy for patients with alcoholic liver cirrhosis. Such results are gradually confirming the safety and efficacy of ABM*i* therapy. Therefore, we believe that ABM*i* therapy represents a promising treatment for advanced liver cirrhosis (Fig. 1).

Peng *et al.* [19▪▪] recruited 527 patients with HBV-related decompensated liver cirrhosis, divided into a group of 53 patients who received 120 ml of autologous bone marrow fluid via a hepatic artery and 105 patients who did not. Results of analysis showed no adverse effects from bone marrow administration.

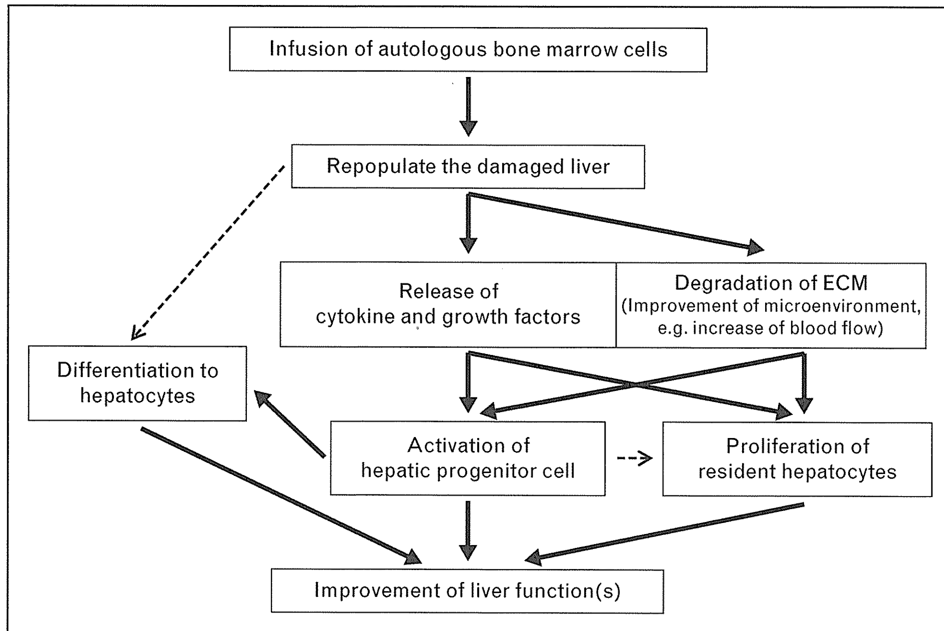

FIGURE 1. Possible mechanism(s) of autologous bone marrow cell infusion therapy. ECM, extracellular matrix.

Patients were divided into a short-term observation group (1–48 weeks) and a long-term observation group (until 192 weeks), and the results of analysis showed improved hepatic function in the early period. Long-term observation showed no change in the incidence of hepatocellular carcinoma (HCC) after the administration of bone marrow cells, suggesting the possibility of an improved survival rate [19■■]. Other studies to date on cell therapies using bone marrow (stem) cells for liver cirrhosis include not only those on our ABM*i* therapy [15,16] but also studies by Lyra *et al.* [20,21■] on the effectiveness of infusion of bone marrow stem cells. Clinical studies by Lyra *et al.* in Brazil suggested the feasibility and safety of ABM*i* through a hepatic artery rather than a peripheral vein for chronic liver disease patients awaiting liver transplantation [20,21■]. In other clinical studies, increased volumes of left lateral hepatic segments were reported by am Esch *et al.* [22,23] with intraportal administration of CD133-positive bone marrow cells after portal venous embolization of right liver segments. Conversely, death due to radiocontrast nephropathy has been reported as a result of infusion of concentrated CD34-positive cells from 200 ml of bone marrow fluid through a hepatic artery into patients with decompensated liver cirrhosis, and that clinical study was discontinued [24]. This indicates the need for clarification of the treatment indication criteria and full investigation of administration routes, cell concentrations and speed of drip infusion. The need for development of less-invasive liver regeneration therapies using cultured autologous bone marrow-derived cells is also suggested.

CULTURED AUTOLOGOUS BONE MARROW-DERIVED CELLS

Our ABM*i* therapy involves bone marrow aspiration under general anesthesia, and is not indicated for patients for whom general anesthesia is difficult. We therefore aimed to develop a new liver regeneration therapy in which cells having a curative effect on liver cirrhosis are isolated and cultured from a small amount of autologous bone marrow aspirated under local anesthesia and infused back into the same patient. The bone marrow contains two major sources of stem cells, hematopoietic stem cells (HSCs) and mesenchymal stem (stromal) cells (MSCs). Previous basic studies suggested the therapeutic effect on advanced liver diseases using these cell types in animal models. Therefore, issues that need to be investigated in the future will include identification of cells showing treatment effects from bone marrow cell fractions, and clarification of the mechanisms underpinning such actions. When cells with liver regeneration and restorative activity can be isolated from small amounts of bone marrow fluid, cultured, and then readministered, the indications would be able to be expanded, as collection of bone marrow fluid would no longer need to be performed under general anesthesia. However, safety evaluation guidelines for cultured cells are needed when autologous cultured bone marrow-derived cells

Table 1. Previous clinical trials in liver disease using autologous bone marrow (stem) cells

Type of infused cells	Number of infused cells	Improvement after the infusion	Number and etiology of patients	Reference
CD34$^+$ cells from G-CSF mobilized peripheral blood	1×10^6–2×10^8	Improved serum albumin and serum bilirubin	Five alcohol	[8]
Only G-CSF mobilization for 5 days	N/A	Elevated serum HGF. Increased hepatic Ki67$^+$/cytokeratin7$^+$ cells	24 alcohol, (11/24 ctrl.)	[9]
Cultured CD34$^+$ cells from G-CSF mobilized peripheral blood	2.3×10^8 (mean)	Improved serum bilirubin and CP	Nine alcohol	[10]
PBMCs from G-CSF mobilized peripheral blood	10^7–10^8/kg	Improved serum albumin and CP	40 HBV (20/40 ctrl., only G-CSF)	[11]
BMNCs from iliac crest (400 ml); ABM*i* therapy	$5.20 \pm 0.63 \times 10^9$	Improved serum albumin and CP	Five HCV, three HBV, one unknown	[15]
BMNCs from iliac crest (500–750 ml) ; ABM*i* therapy	0.48–1.48×10^8/kg	Activation of HPC (cytokeratin7$^+$cells). Increased liver volume	10 HBV	[17■]
		Improved CP		
BMNCs from iliac crest (400 ml); ABM*i* therapy	$8.0 \pm 7.3 \times 10^9$	Improved serum albumin, PT, and CP	10 alcohol (5/10 ctrl.)	[18■]
BMNCs (MSCs) from iliac crest (120 ml)	Not described	Improved serum albumin, bilirubin, PT, and MELD in the early period	158 HBV (105/158 ctrl.)	[19■■]
		No difference of HCC development through 48 weeks		
BMNCs from iliac crest (maximum 50 ml)	At least 1×10^8	Improved serum albumin and bilirubin	10	[20]
BMNCs from iliac crest (maximum 50 ml)	$3.78 \pm 2.69 \times 10^8$	Improved serum albumin, bilirubin, and CP for 60–90 days	30 (15/30 ctrl.)	[21■]
Bone marrow-derived CD133$^+$ cells (60–220 ml)	2.4–12.3×10^6	Increased liver volume after liver resection	Six bearing liver tumors (3/6 ctrl.)	[22]
Bone marrow-derived CD34$^+$ cells from iliac crest (200 ml)	5.25×10^6 (mean) (CD34$^+$, 90.5%)	One case developed radiocontrast nephropathy and then died	One HBV, one PBC	[24]
			One AIH, one cryptogenic	
Cultured bone marrow-derived MSCs (80–100 ml)	31.7×10^6 (mean)	Improved MELD (two of four patients)	Three cryptogenic, one AIH	[28]
Cultured bone marrow-derived MSCs (20 ml)	3–5×10^7	Improved MELD, serum creatinine, prothrombin complex	Four HBV, two unknown	[29]
			One HCV	
Cultured bone marrow-derived MSCs stimulated to hepatic lineage using HGF-containing medium (95 ± 25 ml)	2×10^7 hepatic lineage-committed cells in a total of 2×10^8 cells	Improved MELD and CP	40 HCV (20/40 ctrl.)	[30■]

ABM*i*, autologous bone marrow cell infusion; AIH, autoimmune hepatitis; BMNC, bone marrow mononuclear cell; CP, Child–Pugh score; ctrl., control; G-CSF, granulocyte colony-stimulating factor; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HGF; hepatocytes growth factor; HPC, hepatic progenitor cell; MELD, model for end stage liver disease; MSC, mesenchymal stem cell; N/A, not applicable; PBMC, peripheral blood mononuclear cell; PT, prothrombin time.

are used in liver cirrhotic patients, and a system conforming to SOP at a GMP-grade cell-processing center will be also essential.

Some clinical phase I trials using MSCs have been reported in patients with myocardial infarction [25], amyotrophic lateral sclerosis [26] and Crohn's disease [27]. We can check the current status of clinical trials in this database (*ClinicalTrials.gov*; http://www.clinicaltrials.gov/). About advanced liver disease, Mohamadnejad *et al.* [28] have shown improvements of the model for end-stage liver disease (MELD) score in some patients with peripheral vein administration of cultured MSCs in autologous bone marrow cells. Kharaziha *et al.* [29] also reported that liver function assessed by the MELD score decreased significantly from 17.9 ± 5.6 to 10.7 ± 6.3 after administration of cultured autologous MSCs in four hepatitis B, one hepatitis C, one alcoholic, and two cryptogenic cirrhotic patients. They aspirated around 20 ml of autologous bone marrow fluid from both posterior superior iliac spines under local anesthesia. The mononuclear cells were separated by the Ficoll separation method. Separated bone marrow mononuclear cells were cultured for 2 weeks, and then were collected. They infused about 3×10^7–5×10^7 cells expressing CD44, CD73, and CD105, consistent with MSCs characteristics to the same patient via a portal vein or peripheral vein. Moreover, recently, the clinical trial for patients with hepatitis C virus (HCV)-related liver failure has been reported by Amer *et al.* [30▪]. They also aspirated around 120 ml of autologous bone marrow fluid from the posterior superior iliac crest under local anesthesia, and then injected autologous cultured bone marrow-derived MSCs, which had been stimulated to hepatic lineage using hepatocytes growth factor (HGF) containing medium, into spleen or liver directly using a needle gauge 18 under abdominal ultrasonography guidance. In these patients, the MELD score and Child score were significantly lower than those in the control group from 2 weeks to 6 months after the injection. No difference between intrasplenic route and intrahepatic route was observed.

CONCLUSION

Previous clinical studies using autologous bone marrow cells for advanced liver disease suggested the efficacy and safety (Table 1). However, randomized controlled studies are needed to clearly show the efficacy and safety of liver regeneration therapies using autologous bone marrow (stem) cells or cultured bone marrow-derived cells. And new treatment methods using less-invasive bone marrow-derived cultured cells need to be developed.

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Conflicts of interest

There are no conflicts of interest.

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JB Review

Liver stem/progenitor cells: their characteristics and regulatory mechanisms

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Liver stem cells give rise to both hepatocytes and bile duct epithelial cells also known as cholangiocytes. During liver development hepatoblasts emerge from the foregut endoderm and give rise to both cell types. Colony-forming cells are present in the liver primordium and clonally expanded cells differentiate into either hepatocytes or cholangiocytes depending on culture conditions, showing stem cell characteristics. The growth and differentiation of hepatoblasts are regulated by various extrinsic signals. For example, periportal mesenchymal cells provide a cue for bipotential hepatoblasts to become cholangiocytes, and mesothelial cells covering the parenchyma support the expansion of foetal hepatocytes by producing growth factors. The adult liver has an extraordinary capacity to regenerate, and after 70% hepatectomy the liver recovers its original mass by replication of the remaining hepatocytes without the activation of liver stem cells. However, in certain types of liver injury models, liver stem/progenitor-like cells, known as oval cells in rodents, proliferate around the portal vein, while the roles of such cells in liver regeneration remain a matter of debate. Clonogenic and bipotential cells are also present in the normal adult liver. In this minireview we describe recent studies on liver stem/progenitor cells by focusing on extracellular signals.

Keywords: cytokine/development/differentiation/hepatocyte/regeneration.

Abbreviations: 2-AAF, 2-acetylaminofluoren; AFP, alpha-fetoprotein; ALB, albumin; BMEL, bipotential mouse embryonic liver cell; CDE, choline-deficient, ethionine-supplemented; DDC, 3,5-diethoxycarbonyl-1,4-dihydro-collidine; Dlk, Delta-like protein 1; DPPIV, dipeptidyl peptidase IV; EpCAM, epithelial cell adhesion molecule; FGF, fibroblast growth factor; H-CFU-C, hepatic colony-forming unit in culture; MC, mesothelial cell; OSM, oncostatin M; PH, partial hepatectomy; STM, septum transversum mesenchyme; TNF, tumour necrosis factor; Wt1, Wilms' tumour 1.

The liver is a central organ for homeostasis owing to its numerous functions, including carbohydrate metabolism, glycogen storage, biosynthesis of various biochemical components including amino acids and nucleotides, lipid metabolism, urea synthesis, drug detoxification, production of plasma proteins and hormones, and destruction of erythrocytes. Because the liver is such an essential organ, liver diseases are often fatal. Liver insults such as hepatitis viruses, drugs, alcohol and genetic, metabolic and immune disorders can lead to steatosis, hepatitis, fibrosis, cirrhosis and cancer and liver disease is a major cause of death. The liver is also known as a unique organ that can regenerate, making it possible to transplant the liver from a living donor. However, the molecular mechanisms underlying organogenesis, maintenance, pathogenesis and regeneration of the liver are not well understood. As the liver is a large organ with a variety of functions, it has been used for many decades as a source to purify numerous enzymes for biochemical studies. By contrast, much progress has been made relatively recently in the characterization of each type of liver cell and analysis of their interactions. Those studies have been facilitated by new technologies such as genomics, mouse mutants and the development of various tools to isolate the cells of interest. In this review, we describe recent studies on liver stem/progenitor cells together with the environments that support their proliferation and differentiation during development and pathogenesis.

Liver architecture and liver stem cells

The liver is divided into lobules and each lobule consists of plates of hepatocytes lined by sinusoidal capillaries that radiate towards a central efferent vein (Fig. 1). Liver lobules are hexagonal and at each of six corners there is a portal triad of vessels consisting of a portal vein, hepatic artery and bile duct. Sinusoids are composed of liver-specific capillaries with fenestrated endothelial cells, hepatic stellate cells (Ito cells), liver-resident macrophages (Kupffer cells) and large granular lymphocytes (pit cells). The liver has a dual blood supply, namely, via the portal vein and the hepatic artery. The portal vein delivers the venous blood flowing from the intestines, pancreas and spleen. The hepatic artery supplies oxygen to the liver. The blood flows from a portal triad through a sinusoidal capillary to a central efferent vein. Hepatocytes are major parenchymal cells carrying out most of the metabolic functions and account for ~60% of the total liver cell population and 80% of the volume of the organ. Hepatocytes are highly polarized epithelial cells and

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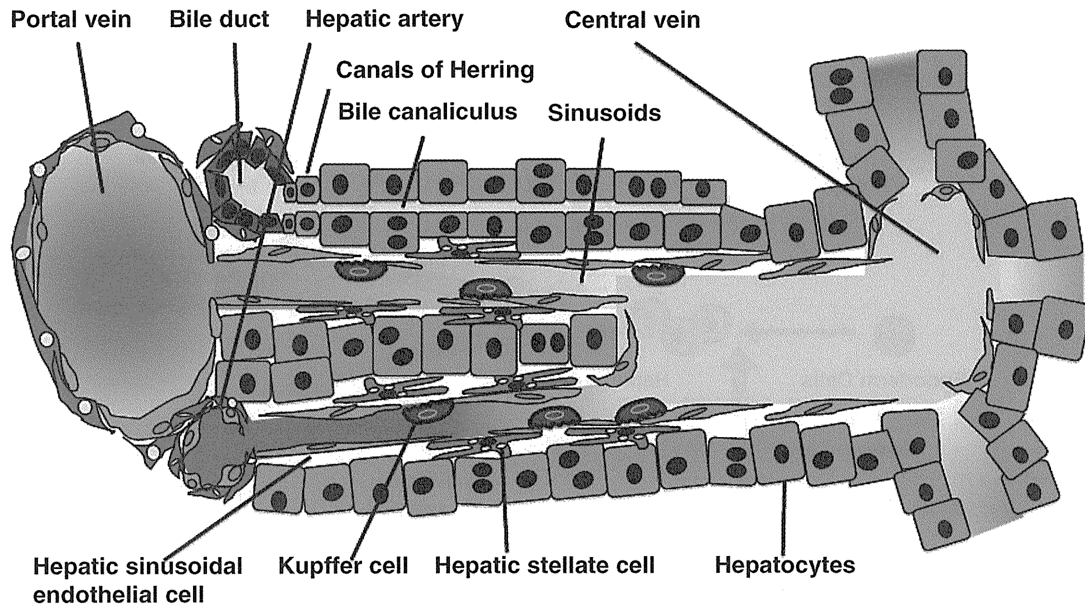

Fig. 1 Liver architecture. In the liver, blood flows from portal blood vessels through sinusoids to central efferent veins. Sinusoids are liver-specific capillaries with fenestrated endothelial cells, hepatic stellate cells (Ito cells) and blood cells such as liver-resident macrophages (Kupffer cells). Hepatocytes are highly polarized epithelial cells forming cords, and plates of hepatocytes are lined by sinusoidal capillaries that radiate towards a central efferent vein. Tight junctions formed between hepatocytes create a canaliculus that surrounds each hepatocyte. Bile salts produced in hepatocytes are excreted into canaliculi that are linked to bile ducts. The region that connects the bile canaliculus and the biliary tree is called 'canals of Hering'.

form cords. Their basolateral surfaces face fenestrated sinusoid endothelial cells, facilitating the transfer of materials between hepatocytes and blood flows. Tight junctions formed between hepatocytes create a canaliculus that surrounds each hepatocyte. Bile salts produced in hepatocytes are excreted into canaliculi that are linked to bile ducts at the portal triad. Bile ducts are formed by a specialized type of epithelial cell called a biliary epithelial cell or a cholangiocyte.

In general, stem cells are characterized by their ability to self-renew and differentiate to multiple lineages. As hepatocytes and cholangiocytes, the two types of liver epithelial cells, are derived from a common origin during organogenesis, those cells with the potential to proliferate and give rise to both types of liver epithelial cells are considered to be liver stem cells. Although there are many reports describing liver stem cells, the definitions of stem cells are rather vague in many of them. As it is not an easy task to distinguish stem cells from progenitors because of the difficulty of proving the unlimited self-renewal activity of stem cells in many situations, we use the term stem/progenitor cells to describe such cells in this review article.

The onset of liver development

Liver organogenesis begins at embryonic day (E) 8.5 in the mouse from the foregut endoderm. The ventral wall of the foregut endoderm faces the developing heart by approximately E8 and receives inductive signals for hepatic fate, such as fibroblast growth factor (FGF) from the heart (*1–3*) and bone morphogenetic protein from the septum transversum mesenchyme (STM) (*4*). Wnt2b is expressed in the lateral plate mesoderm adjacent to the endoderm destined to be the liver and is essential for the onset of liver development in zebrafish (*5*). By these signals, hepatoblasts emerge from the foregut endoderm and migrate as cords into the surrounding STM (*6, 7*). Analysis of Flk1-deficient mouse embryos revealed that $Flk1^{+}$ endothelial cells are required for proliferation of hepatoblasts (*8*). Because hepatoblasts proliferate and give rise to both hepatocytes and cholangiocytes as described below, they are considered to be embryonic liver stem/progenitor cells.

Identification and characterization of hepatoblasts

As cell sorting using antibodies is a powerful means to isolate and characterize a specific cell type, efforts have been made to search for specific cell surface antigens on hepatoblasts (Fig. 2). Kubota and Reid (*9*) showed that the $RT1A1^{-}$ $OX18^{low}$ ICAM-1^{+} fraction of E13 rat foetal liver contained hepatoblasts. Suzuki *et al.* (*10*) developed a single cell-based assay designated the hepatic colony-forming unit in culture (H-CFU-C) and showed that the $CD45^{-}$ $TER119^{-}$ c-Kit^{-} $CD29^{+}$ $CD49f^{+}$ and $CD45^{-}$ $TER119^{-}$ c-Kit^{-} c-Met^{+} $CD49f^{+/low}$ fraction of E13.5 mouse liver contained hepatic progenitor/stem cells. They also showed that $CD45^{-}$ $TER119^{-}$ c-Kit^{-} c-Met^{+} $CD49f^{+/low}$ cells of E11.5 mouse liver had high H-CFU-C potential and that clonally expanding cells reconstituted the liver, pancreas and intestine *in vivo*. On the other hand, Minguet *et al.* (*11*) reported that $CD45^{-}$ $TER119^{-}$ c-Kit^{low} cells in E11 mouse liver contained the earliest hepatic progenitors, also displaying features of

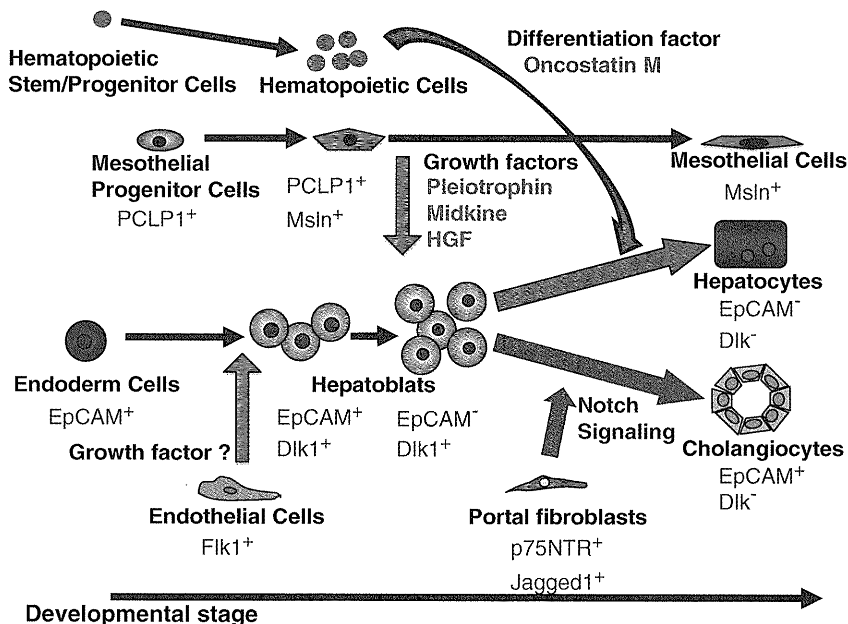

Fig. 2 Development of liver cells and expression of cell surface markers. The EpCAM$^+$DLK1$^+$ hepatoblasts emerge from EpCAM$^+$ foregut endoderm cells and form liver primordium. Then, the hepatoblasts dramatically reduce the expression of EpCAM. EpCAM is upregulated again in biliary epithelial cell precursor cells around the portal vein, where p75NTR$^+$ Jagged1$^+$ portal fibroblasts interacted with hepatoblasts. PCLP1$^+$ mesothelial progenitor cells produce growth factors for hepatoblasts to proliferate. OSM secreted by haematopoietic cells induces hepatocytic differentiation of hepatoblasts.

liver-repopulating stem cells. Delta-like protein 1 (Dlk1), also known as Pref-1, was strongly expressed in liver buds as early as E10.5 in mice. Dlk1$^+$ cells isolated from E14.5 livers expressed albumin (ALB) and formed colonies composed of the hepatocyte and cholangiocyte lineages in the presence of hepatocyte growth factor and epidermal growth factor, indicating that liver stem cell activity is present in this population (*12*). As in mouse foetal liver, Dlk1 is also expressed strongly in human foetal liver (*13*, *14*). Nierhoff *et al.* showed that murine foetal liver alpha-fetoprotein (AFP)$^+$/ALB$^+$ cells were positive for Dlk1 and E-cadherin and that purified E-cadherin$^+$ epithelial cells formed clusters in cell culture and differentiated along the hepatocytic lineage. Interestingly, AFP$^+$/E-cadherin$^+$ epithelial cells were Sca-1$^+$, but showed no expression of c-Kit. In order to examine their *in vivo* capacity, wild-type E12.5 mouse liver epithelial cells were transplanted into adult dipeptidyl peptidase IV (DPPIV) knockout mice, and DPPIV expression was used as a marker to discriminate the donor from recipient cells. This resulted in incorporation of the DPPIV$^+$ donor-derived cells into the hepatic parenchymal cords of the recipient liver, showing a repopulation and differentiation capacity of the E12.5 E-cadherin$^+$ cells (*15*).

Epithelial cell adhesion molecule (EpCAM) is expressed in HNF4α^+ hepatoblasts of liver buds as early as E9.5 in mice (Fig. 2). Colony-forming assays using sorted E11.5 liver cells revealed that the EpCAM$^+$ Dlk1$^+$ cell population contained *in vitro* colony-forming cells, indicating that liver stem cell activity is present in this population. EpCAM expression declined by E13.5 in mouse liver, while Dlk1 expression was sustained by E16.5 (*16*). In humans, Dan *et al.* (*17*) reported that multipotent progenitor cells derived from human foetal liver expressed EpCAM,and Schmelzer *et al.* (*18*) reported that pluripotent precursors of hepatoblasts expressed EpCAM and were located in ductal plates in human foetal liver. CD13 (aminopeptidase N) was detected on the cells of the Dlk1$^+$ hepatic stem/progenitor fraction. Colony formation assays revealed that hepatic stem/progenitor cells were enriched in the CD13$^+$ fraction, compared with the Dlk1$^+$ fraction, of non-haematopoietic cells in foetal liver (*19*).

Characteristics of foetal liver stem/progenitor cells

Dlk1$^+$ cells contain some clonogenic cells named hepatic progenitor proliferating on laminin that continuously proliferate on laminin-coated plates and differentiate to both hepatocytes and cholangiocytes depending on culture conditions, suggesting that they are liver stem cells (*20*). Bipotential cell lines, referred to as bipotential mouse embryonic liver cell (BMEL), were also obtained after a long latency in culture of foetal liver cells and they were shown to give rise to both hepatocytes and cholangiocytes in recipient mice, although the origin of BMEL was unknown (*21*). These cell lines are used to study the mechanisms of hepatocytic and/or cholangiocytic differentiation from liver stem cells.

In the past decade, a number of cell surface markers for foetal liver cells have been found and used to prospectively isolate and to localize them in the liver. While some studies used transplantation assays to

investigate the repopulation capacity, the ability to form a colony and differentiate to both lineages *in vitro* is a practical criterion to evaluate hepatoblasts in most of these studies. In the case of haematopoietic stem cells, a single purified stem cell can be shown to propagate and give rise to all kinds of haematopoietic cells for the long term in an irradiated recipient mouse, providing clear evidence for stemness *in vivo*, that is self-renewal ability and multi-lineage differentiation. By contrast, as liver repopulation assays require a large number of cells to be transplanted to demonstrate engraftment capacity, rigorous proof of stemness *in vivo* is difficult. Nonetheless, there is little doubt that hepatoblasts possess capacities of liver stem cells on the basis of numerous previous works as described above (*9–12*, *15–19*).

Differentiation of hepatoblasts to cholangiocytes

Bile ducts are formed only around the portal vein, suggesting that regionally specific signals induce cholangiocytes from hepatoblasts. Indeed, two signalling pathways, TGFβ/Activin and Notch, are specifically activated in hepatoblasts near the portal vein. TGFβ2 and TGFβ3 are predominantly expressed in the portal region (*22*), and the Onecut family of transcription factors, HNF6 (OC-1) and OC-2, promote expression of α2-macroglobulin and follistatin, inhibitors of the TGFβ/Activin pathway, in the parenchymal region (*23*). Dlk1$^+$ hepatoblasts express Notch2, whereas p75NTR$^+$ periportal fibroblasts express Jagged-1 (*24*). Forced expression of Notch intracellular domain in Dlk1$^+$ hepatoblasts resulted in differentiation to cholangiocytes (*25*). These results strongly suggest that cholangiocyte differentiation is induced by Notch signalling in the periportal region. Although differentiation of hepatoblasts to cholangiocytes by TGFβ and Notch signalling occurs in mid-gestation, surprisingly, hepatocytes turned to cholangiocytes and formed ectopic duct structures in the parenchyma by Notch activation after birth (*26*). These results indicate that not only hepatoblasts but also hepatocytes are competent to differentiate to cholangiocytes at least by the neonatal period (Fig. 2).

Immature cholangiocytes form a ductal plate, a single cell layer, around the portal vein. Tubular morphogenesis of bile ducts proceeds through the rearrangement of a single layer of the ductal plate. Recent studies on mice lacking Sox9, a transcription factor, or Notch 2 in the liver indicated the second wave of cholangiocyte differentiation adjacent to the initial single layer of the ductal plate, which was regulated by TGFβ and Notch pathways and involved in tubular morphogenesis. In a model proposed on the basis of those studies, after the initial induction of cholangiocytes near the portal vein, cholangiocyte differentiation and tubular morphogenesis progress in parallel (*22*, *26–30*). However, the precise mechanisms of bile duct morphogenesis have not been completely understood.

In addition, studies using mutant mice have implicated transcription factors including HES1, HNF6, HNF1β, Tbx3, FoxA2 and A3, FoxM1b, Hex and Sall4 in bile duct differentiation and/or morphogenesis (*31–38*) (Fig. 3). Although a network of these transcription factors and a link between transcription factors and Notch/TGFβ pathways are being uncovered (*39*), studies on gene expression and histology of mutant mice are insufficient to understand how these factors regulate complicated processes of tubulogenesis. As an alternative approach, *in vitro* culture systems allowing hepatoblasts to form bile duct structures are helpful to understand the lineage commitment of hepatoblasts and tubular morphogenesis (*22*, *25*, *40*, *41*).

Proliferation and differentiation of hepatocytes in foetal liver

At an early stage of hepatogenesis, endothelial cells contribute to the proliferation of hepatoblasts (*8*) and the vast majority of hepatoblasts become parenchymal hepatocytes at a later stage. The liver parenchyma is covered with the mesothelium consisting of the surface mesothelial cell (MC) layer, ALCAM$^+$ submesothelial cells and fibroblasts (*42*). At a later stage of hepatogenesis, MCs seem to contribute to the expansion of hepatoblasts (*43*). Foetal liver MCs are characterized by the expression of a sialomucin, PCLP1, and become adult liver MCs expressing mesothelin. Comparison of the gene expression profiles between foetal and adult MCs revealed that foetal PCLP1$^+$ MCs express various growth factors for hepatocytes such as Midkine and Pleiotrophin, and co-culture of Dlk1$^+$ foetal hepatocytes with PCLP1$^+$ foetal MCs in a transwell enhanced hepatocyte proliferation. Wilms' tumour 1 (Wt1) knockout mice were embryonic lethal, exhibiting impaired liver development. Cytokine production by Wt1 knockout MCs was reduced, while proliferation of Dlk1$^+$ cells from Wt1 knockout embryos was normal in a co-culture with wild-type MCs, indicating that defects in liver development of Wt1

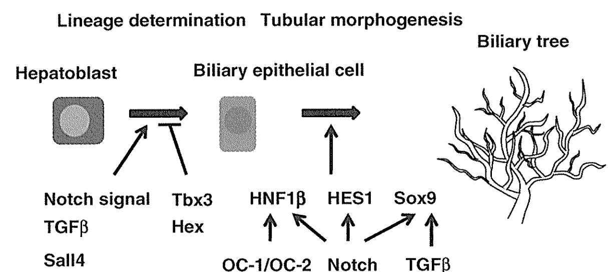

Fig. 3 Bile duct development. There are two steps in bile duct development. First, hepatoblasts are induced to differentiate to biliary epithelial cells around the portal vein. This step is promoted by TGFβ and Notch signals as well as a transcription factor, Sall4, whereas it is inhibited by two transcription factors, Tbx3 and Hex. Biliary epithelial cells then undergo tubular morphogenesis and form the biliary tree. Three transcription factors, HNF1β, HES-1 and Sox9, are involved in tubular morphogenesis. The Notch signal is upstream of all the three transcription factors, whereas OC-1 and OC-2, and the TGFβ signal are upstream of HNF1β and Sox9, respectively.

knockout mouse are due to MCs. MCs were also shown to delaminate and give rise to mesenchymal cells in the liver (*44*). These results indicate that the mesothelium is not only a protective sheet covering the liver parenchyma but also actively involved in liver organogenesis (Fig. 2).

Foetal liver is a major tissue for haematopoiesis, and hepatocytes acquire various metabolic functions at perinatal and postnatal stages. Mice lacking gp130, the common receptor subunit of the IL-6 family cytokines, develop liver with impaired functions, indicating that some of the IL-6 family cytokines are required for functional maturation of the liver (*45*). Oncostatin M (OSM), a member of the IL-6 family, strongly enhanced differentiation of foetal hepatocytes, while liver development is normal in OSM-deficient mice, suggesting that another member of the family may play a similar role. In the foetal liver, immigrating haematopoietic stem cells proliferate and produce numerous blood cells with the help of liver cells including hepatocytes and endothelial cells. Haematopoietic activity in foetal liver declines with hepatocyte differentiation (Fig. 2). As OSM is secreted from haematopoietic cells proliferating in the foetal liver and induces differentiation of hepatocytes, it is likely that OSM plays a role for coordination of liver development and haematopoiesis (*46*).

Adult liver stem/progenitor cells

Adult liver has a potential to regenerate under conditions of severe parenchymal loss, although hepatocytes and cholangiocytes are mitotically dormant under normal conditions. Hepatocytes themselves have a remarkable ability to self-replicate to restore liver mass (*47*) and are capable of at least 80 doublings by serial transplantation (*48*), allowing the liver to regenerate. Thus, the contribution of liver stem cells to regeneration after partial hepatectomy (PH) seems to be minimal if any. However, in liver injury that limits this pathway there is an accompanying expansion of a potential stem cell compartment in the periportal area, which is known as ductular reaction (*49–51*) (Fig. 4). These proliferating epithelial cells are often referred to as oval cells in rodents because of their oval nucleus (*52*). Upon activation of oval cells, they expand into liver parenchyma from the portal area, and selective damage of the periportal zone reduces oval cell proliferation, supporting the notion that oval cells are derived from the periportal region, in particular canals of Hering that connect the bile canaliculus and the biliary tree (*53*). In addition, an extrahepatic origin of oval cells such as bone marrow was also suggested (*54*); however, the exact origin of oval cells still remains to be established. While oval cells have been most extensively studied in rodents, similar cells have been found in various human liver diseases, such as chronic viral hepatitis, alcoholic liver disease, nonalcoholic fatty liver disease and fulminant hepatitis, and also implicated in tumourigenesis (*55*, *56*). Oval cells express both ALB and cytokeratin 19, which are hepatocytic and cholangiocytic markers, respectively, and are believed to differentiate to hepatocytic and biliary lineages, similar to hepatoblasts in the embryonic liver.

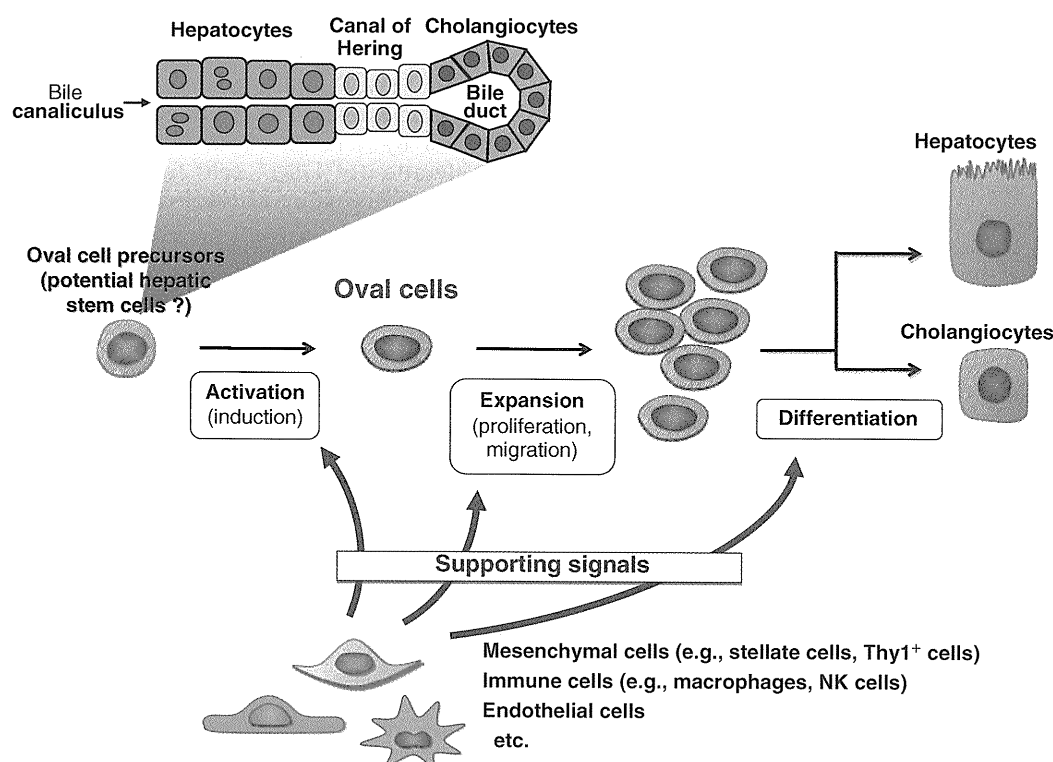

Fig. 4 Induction of oval cells. Oval cells and $Thy1^+$ cells are simultaneously induced in severe liver damage conditions. The origin of oval cells is still under debate. FGF7 is produced by $Thy1^+$ cells in response to liver damage conditions, and oval cells receive its signal.

Thus they are thought to be facultative stem/progenitor cells in the adult liver (Fig. 4).

The nature of oval cells as liver stem cells was debated in numerous reports of studies using various rodent models. The 2-acetylaminofluoren (2-AAF)/PH model, *i.e.* blocking hepatocyte proliferation by 2-AAF prior to PH, has been extensively used to characterize oval cells in rat (*57, 58*). However, the same procedure does not induce oval cells in mice, and alternative protocols such as a choline-deficient, ethionine-supplemented (CDE) diet and 3,5-diethoxy-carbonyl-1,4-dihydro-collidine (DDC) diet have been developed to induce oval cells in mice (*59–61*). Although the proliferating epithelial cells in the periportal region upon injury by various insults are collectively referred to as oval cells, it remains unclear whether or not the oval cells in different species by different protocols have common characteristics. A major problem in characterizing oval cells was the lack of appropriate cell surface markers to identify and isolate the oval cell compartment.

In the rat 2-AAF/PH model, Dabeva *et al.* reported that $EpCAM^+$ oval cells are bipotential adult hepatic progenitors (*62, 63*). Suzuki *et al.* (*64*) reported that $CD133^+$ cells isolated from DDC-treated mouse liver could form large colonies in culture. These large colony-forming cells gave rise to both hepatocytes and cholangiocytes, while maintaining undifferentiated cells by self-renewing cell divisions. In order to isolate and characterize mouse oval cells, Okabe *et al.* (*65*) searched for cell surface molecules expressed on oval cells in mouse fed DDC diet. EpCAM was expressed in both mouse normal cholangiocytes and oval cells, and its related protein TROP2 was expressed exclusively in oval cells, establishing TROP2 as a novel marker to distinguish oval cells from normal cholangiocytes (*65*). Some of the $EpCAM^+$ cells isolated from injured liver proliferate to form colonies *in vitro*, and the clonally expanded cells differentiate into hepatocytes and cholangiocytes, suggesting that the oval cell fraction contains potential liver stem cells.

Interestingly, such cells with liver stem cell characteristics are also found in $EpCAM^+$ cells of the normal liver. Intriguingly, comparison of the colony formation of $EpCAM^+$ cells between normal and injured livers revealed little difference in the frequency of potential liver stem cell activity between them, strongly suggesting that most of the proliferating mouse oval cells represent transit-amplifying cells rather than stem cells. Bipotential clonal cell lines can be obtained from the healthy liver of adult mice and participate in liver regeneration in severe combined immune-deficient mice expressing urokinase-type plasminogen by the ALB promoter, where they differentiate in clusters of hepatocytes and occasionally bile ducts (*66*). Kamiya *et al.* (*67*) found progenitor cells in the $CD13^+CD49f^+CD133^+$ subpopulation of non-haematopoietic cells derived from postnatal livers. These results demonstrate the existence, in normal adult mouse liver, of a pool of clonogenic cells that are (or can become) bipotential.

As mentioned above, oval cells are induced in liver with severe or chronic damage. Chronic injury conditions in the liver are usually associated with inflammation, and the roles of lymphocytes and inflammatory responses in oval cell regulation have also been suggested (*68, 69*). In accordance with this notion, several kinds of inflammatory cytokines, such as tumour necrosis factor (TNF)-α, lymphotoxin-β, interferon-γ and IL-6, have been shown to modulate oval cell response (*70, 71*). Perhaps the best established inflammatory cytokine to be involved in oval cell response is a TNF family member ligand, TNF-like weak inducer of apoptosis (Tweak). Thus, transgenic mice overexpressing this cytokine in the liver exhibit periportal oval cell hyperplasia, while administration of a blocking anti-Tweak monoclonal antibody significantly reduced oval cell response in mice fed DDC diet (*72*). Furthermore, in mice lacking Fn14, the cognate receptor for Tweak, induction of oval cells was attenuated in both DDC diet and CDE diet models (*72, 73*). These inflammatory cytokines are considered to function as part of the innate immune system sensing damage to the tissue and serve as the earliest signals for triggering the process of liver regeneration (Fig. 4).

Mesenchymal cells such as stellate cells have long been suggested to physically interact with oval cells and thus considered to induce some signals in them (*53*). Recent studies using several rat and mouse models have demonstrated that a population of mesenchymal cells expressing thymus cell antigen-1 (Thy-1; also known as CD90) resides in close proximity to and expands in parallel with oval cells (*74*) (H. Takase, T. Itoh and A. Miyajima, unpublished observation). Furthermore, these $Thy1^+$ cells were found to express FGF7, and its cognate receptor FGFR2b was detected in oval cells. FGF7 knockout mice showed a defect in oval cell response, while overexpression of FGF7 *in vivo* in normal mouse liver led to induction and proliferation of cells with markers of oval cells in the periportal area. Together, these results strongly suggest that FGF7 plays a key role in adult liver stem/progenitor cell response as well as that the $Thy1^+$ cells may serve as the niche for oval cells by providing this cytokine (Takase, H., Itoh, T. and Miyajima A., unpublished observations). As a signal related to oval cell response, several recent studies have implicated the canonical Wnt/β-catenin pathway in oval cell regulation (*75–78*) (Fig. 4). The Wnt/β-catenin pathway is well known to play important roles in stem cell regulation including self-renewal in various other organs and tissues, and also in carcinogenesis including liver tumours. In both rat and mouse models, expression of some Wnt ligands in damaged liver and concomitant activation of the β-catenin pathway in oval cells were observed. In conditional knockout mice lacking β-catenin in both hepatocytes and cholangiocytes, DDC diet-induced oval cell response in the liver was significantly reduced, although not completely abrogated. While several factors have been shown to be involved in oval cell response, the precise modes of their actions and their relationship are currently unclear and should be determined.

Concluding remarks

Traditionally, research on liver biology mostly relied on relatively crude cell separation methods based on cell density and centrifugation. In the last decade, identification of specific cell surface markers for each of the liver cell types, production of corresponding monoclonal antibodies and cell sorting techniques have together revolutionized the field and enabled us to perform much more detailed characterization of liver cells, particularly non-parenchymal cells including the stem/progenitor cells. It has also become possible to analyse the modes of interaction among different types of these cells *in vivo* by means of combinatorial use of specific markers/antibodies as well as *in vitro* with co-culture systems using the isolated viable cell populations. Elucidation of the molecular basis for the signals that regulate development, proliferation and differentiation of liver stem/progenitor cells should not only advance our understanding of the basic pathophysiology of the liver but also help to establish better protocols to generate mature hepatocytes and other liver cells *in vitro* for cell-based therapy, transplantation and drug discovery.

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Conflict of interest

None declared.

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Chapter 14
Liver Stem Cells

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Abstract The liver is an essential organ for life, serving as the center for metabolism and playing various critical functions in controling systemic homeostasis. Among multiple types of cells comprising the liver, hepatocytes and cholangiocytes are the two epithelial cell lineages in the organ and commonly originate from hepatoblasts during organogenesis in the developing embryos. Thus, hepatoblasts possess bi-lineage differentiation potential into hepatocytes and cholangiocytes, a phenotypic feature that can best distinguish and define liver stem cells. Although the liver is considered not to rely on any resident stem cell population for their homeostatic maintenance, facultative stem/progenitor cells with the bi-lineage differentiation potential, referred to as oval cells in rodents, do emerge under severe damage conditions and contribute to the regenerative processes. Identification of specific markers has enabled researchers to isolate and characterize these fetal and adult stem/progenitor cell populations. *In vitro* culture systems as well as *in vivo* studies using animal models have been elucidating detailed molecular mechanisms, including intercellular signaling webs and intracellular transcriptional regulatory networks, that coordinately regulate development, differentiation and behavior of these cells. Understanding the cellular and molecular basis of liver development and regeneration from the perspective of the embryonic and adult stem/progenitor cells should make invaluable contributions to future development of technologies to produce fully functional hepatocytes *in vitro* that are applicable for cell therapy and pharmaceutical screening.

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