the lineage commitment of hepatocytes is not fully understood. The effects of stem cells on liver fibrosis were also analyzed by another group.[12][13] Bone marrow cell infusion and mesemchyamal stem cell infusion were found to improve liver fibrosis in another mouse model. Based on these studies, bone marrow cell infusion appears to improve the microenvironment in the cirrhotic liver.[8] This reparative mechanism was important for development of ABM*i* therapy for LC patients.

Clinical study: ABM*i* therapy for LC patients

We started a clinical trial on ABM*i* therapy for LC patients in November 2003 (Fig. 2). Subjects were LC patients with total bilirubin (TB) <3.0 mg/dL, platelets (Plt) >5 (10^{10}/L) and no viable hepatocellular carcinoma on diagnostic imaging. Autologous bone marrow (400 mL) was isolated from the ilium under general anesthesia. Mononuclear cells (MNC) were separated by cell washing and were infused via the peripheral vein. MNC characteristics were confirmed by fluorescence-activated cell sorter (FACS) analysis (CD34, CD45, c-kit). After ABM*i* therapy, liver function was monitored by blood examination for 24 weeks. From 400 mL of BM, we obtained MNC, and these were infused into LC patients. We then monitored liver function using ultra-sonography, computed tomography (CT) and laboratory tests.

Significant improvements in serum albumin levels and total protein were seen at 24 weeks after ABM*i* therapy ($P < 0.05$). Child-Pugh score improved significantly at 4 weeks and 24 weeks after ABM*i* therapy ($P < 0.05$). In addition, AFP and PCNA expression in liver biopsy tissue was significantly elevated after ABM*i* therapy ($P < 0.05$). No severe adverse effects were observed.[1]

A multicenter trial of ABM*i* therapy in Japan was also carried out at Yamagata University beginning in February 2006. At Yonsei University in Korea, the Yamaguchi-Yonsei collaboration study for ABM*i* therapy started in November 2006 (Fig. 2). In these studies, the safety and effectiveness of ABM*i* therapy were confirmed. In India and Brazil, cell therapy using BMC for LC patient has also been studied, and its effectiveness has been confirmed.[14]

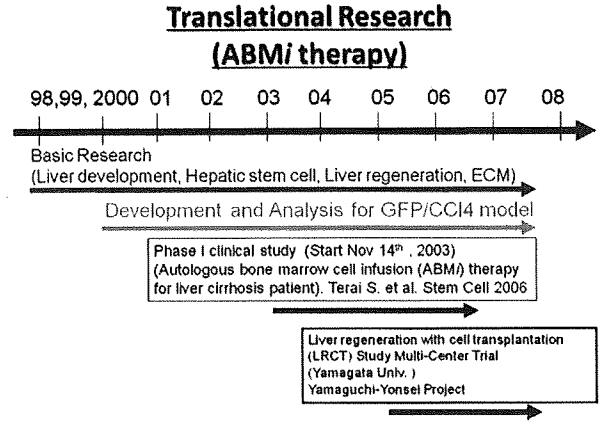

Fig. 2 Time line of translational research for ABM*i* therapy.

Future prospects

Based on previous clinical studies, we found that cell therapy using autologous bone marrow cell is safe and effective for LC patients.[1][2] Recently, in Iran, a similar study was performed and the effectiveness of cell therapy using BMC was also confirmed.[15][16] Although the mechanisms of stem cell differentiation within the human liver remain unclear, therapy using BMC has great potential for LC patients. A randomized multi-center clinical study is now needed for further application of ABM*i* therapy in LC patients.

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Editorial

Fish model leads to new findings in liver disease

See article in *Hepatology Research* 39: 633–644
Liver development: lessons from knockout mice and mutant fish
Takashi Nakamura, Hiroshi Nishina

In liver research, rodent models have primarily been used to identify disease mechanisms, diagnostic criteria and therapies. Tissue-specific knockout systems have also recently been developed. On the other hand, small fish such as zebrafish (*Danio rerio*)[1] and medaka (*Oryzia laptipes*) have been applied in new research models.[2,3] Medaka and zebrafish compare favorably to rodents as experimental animals for drug screening because medaka and zebrafish have a high reproductive rate, mature rapidly and cost little in terms of rearing space and daily maintenance due to their small size.

In a previous issue of the Journal, Nakamura *et al.* reported a comparative analysis of mice and medaka with regard to liver development findings.[2] In the field of liver development, mouse models were analyzed using a reverse genetic approach to identify phenotypic changes using specific gene targets.[2] Various genes, including *BMP4*, *Hhex*, *Xbp1*, *NF-κβ* and *c-jun*, were found to be important in the generation and differentiation of hepatoblasts, and new monoclonal antibodies have been found to be useful in monitoring lineage commitment during hepatocyte differentiation. Liv8/CD44 are particularly useful markers in understanding hepatoblast differentiation.[4–6]

On the other hand, medaka could be applied to forward genetic screening using N-acethyl-N-nitrosourea (ENU) treatment.[2,3] Previous forward ENU screening in Japan has established five groups of medaka liver mutants, while 19 medaka liver mutants have been established and their mutant genes are now being analyzed. In addition, 15 types of zebrafish liver mutants have been established.[1] The merit of forward genetic screening is powerful because forward screening is an unbiased approach to identify genes essential for the process of liver development.[1] In zebrafish and medaka, specific gene analysis for early development through morpholine knockdown has been shown to be effective.[7] TILLING[8] and zinc finger nucleases have recently been developed to find target gene mutants in zebrafish and medaka.[9]

We believe that new systems for drug screening using medaka and zebrafish will greatly assist in the search for new candidate drugs. Previous research using medaka and zebrafish has focused on developmental biology, but there remains an urgent need for research concerning the mechanisms and treatment of liver disease. In Japan, the history of medaka research began with a hepatocellular carcinoma model developed in the 1980s.[10] Disease models such as liver tumors in zebrafish combined with ultrasonography[11] and a non-alcoholic steatohepatitis (NASH) medaka model[12] have also recently been developed, and these models will be useful for drug screening.

We have proposed two drug screen methods using medaka and zebrafish. As shown in Figure 1(a), medaka and zebrafish liver mutants were used to screen new candidate compounds to reduce the deleterious effects of mutant genes in liver mutants. Embryos of liver mutants were bred on culture plates with various chemical compounds. Any specific chemical compounds that allow liver mutants to grow normally would thus be candidates to compensate for the effects of mutant genes. This drug screening system is specific for identifying candidate drugs for liver development. An alternative system is shown in Figure 1(b); wild-type and specific mutants showing liver disease,[3] and medaka and zebrafish with specific promoter transgenes are bred and raised on specific diets, such as high-fat, choline-deficient or N-nitrodiethylamine (DEN)-containing diets. Combination studies using liver mutants and TG

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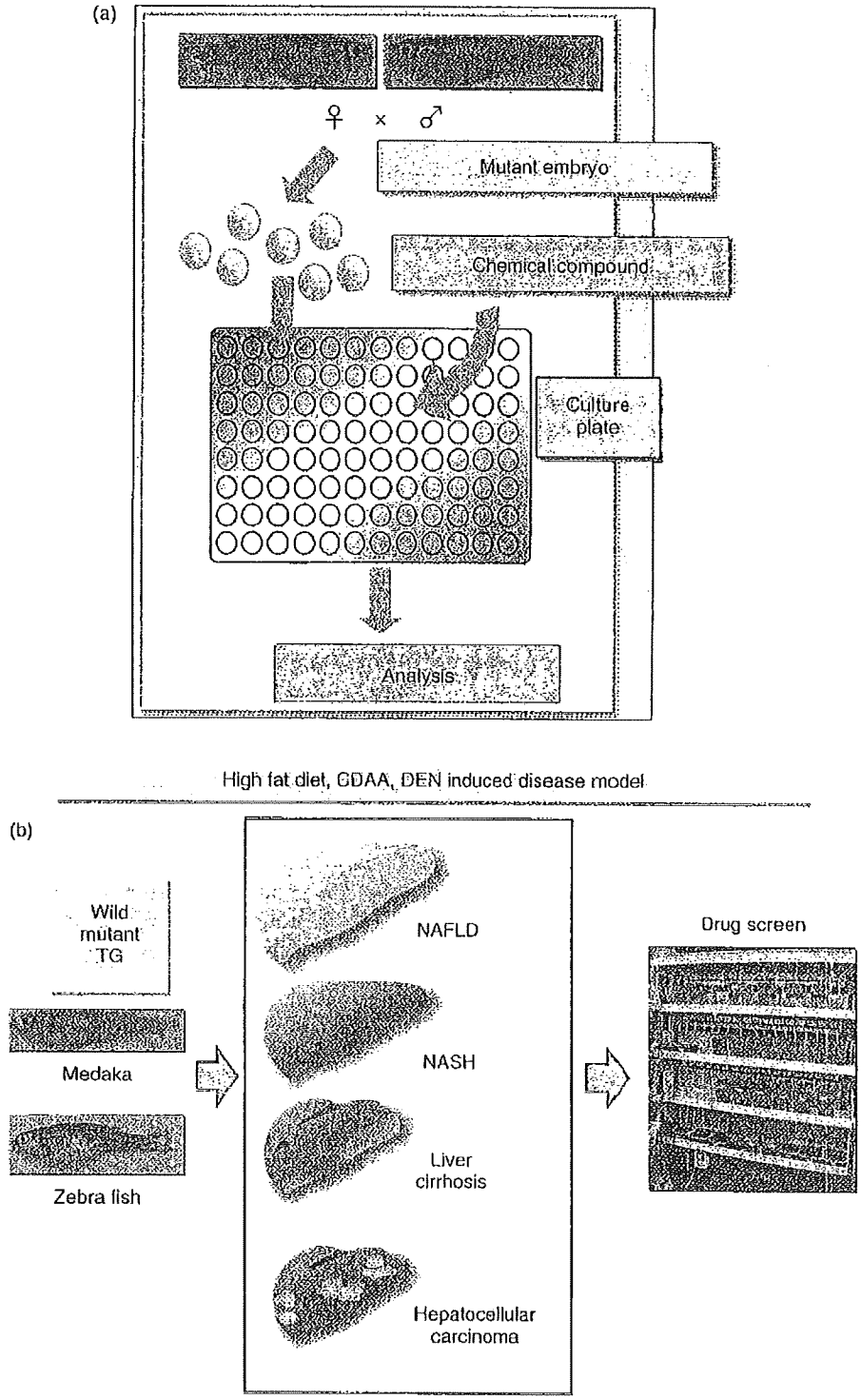

Figure 1 Drug screening using medaka and zebrafish. NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

fish with specific diets are thus useful. Using this system, candidate drugs for NASH, liver cirrhosis and hepatocellular carcinoma will be developed. Sequencing of the medaka and zebrafish genome has been completed and techniques for producing transgenic and knockout animals have been established.[1,13,14] Thus, an increasing number of genetic mechanisms can now be analyzed more efficiently.

At present, new approaches should be developed in order to incorporate the new techniques in medaka and zebrafish research. Fish models will readily allow new findings to be translated into clinical research.

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Diagnosis and treatment of portal hypertension

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Major progress has been made in the area of diagnosis and treatment of portal hypertension. Multi-detector row computed tomography (MD-CT) enabled the revelation of the precise overview of portal hemodynamics and was applied into the decision of therapeutic strategies. A noninvasive liver stiffness measurement device, transient elastography, is expected to be applied in the prediction of existence of esophageal varices. Balloon-occluded retrograde transvenous obliteration (B-RTO) is performed mainly in Japan to eradiate the fundic varices and high eradiation rate and low recurrence rate are reported. Partial splenic embolization (PSE) has been performed to improve hypersplenism without serious side-effect and excellent result is reported. Angiotensin receptor blockers (ARBs) are promising drugs for its portal hypotensive effect and antifibrotic effect and more investigation is necessary. Anti-fibrotic therapy, including autologous bone marrow therapy, has the possibility to improve not only liver fibrosis but also portal hypertension.

Key words: portal hypertension, balloon occluded transvenous obliteration, partial splenic embolization, anti fibrotic therapy, autologous bone marrow cells infusion, angiotension II receptor type 1 blockers

INTRODUCTION

PORTAL HYPERTENSION IS a major complication of cirrhosis, which contains symptoms such as esophageal gastric varices, ascites, hepatic encephalopathy, and hepatorenal syndrome. Portal hypertension is defined as an increase in portal blood pressure and is estimated by the hepatic venous pressure gradient (HVPG), which is the gradient between wedged hepatic venous pressure and free hepatic venous pressure. Portal hypertension is defined as a state where portal vein pressure increased more than 200 mmH_2O, which is equivalent of 14.7 mmHg according to the Japanese guideline.[1]

Acute variceal bleeding is the most life-threatening complication of portal hypertension, and the prevention of variceal formation and its rupture is extremely important. Formation of porto-systemic collaterals develops when portal pressure rises above 10 mmHg[2] and bleeding from varices occurs when pressure rises above 12 mmHg.[3] Therefore, a reduction of the portal vein pressure less than 12 mmHg is considered to be a target pressure in the pharmacological therapy in portal hypertension.[4] Although it is difficult to measure HVPG repeatedly because of its invasiveness, non-invasive diagnostic tool, such as transient elastography (FibroScan, Echosense, Paris), which is a device to measure liver fibrosis, has been applied to predict esophageal varices.[5–8] In addition, Multi-detector row computed tomography (MDCT) angiography is a minimally invasive technique for imaging fundal varices, as well as afferent and efferent veins, and provides beneficial anatomical information for deciding the therapeutic strategies and for evaluation of the effect of treatment.[9,10]

DIAGNOSIS OF PORTAL HYPERTENSION BY NON-INVASIVE PROCEDURE

DIAGNOSTIC TOOLS FOR assessment of portal hypertension are classified into several categories; (i) Estimation of portal vein pressure by measuring HVPG, (ii) Imaging diagnosis of portal hemodymanics, ascites, and splenomegaly by MDCT, abdominal ultrasonography, or angiography, (iii) Endoscopic diagnosis of grading of esophageal varices.

Measuring HVPG is the appropriate method to assess portal vein pressure and is utilized in the judgment of indication or evaluation of the effect of drug therapy for the portal hypertension. Although it is not performed

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repeatedly because of its invasiveness, the development of alternative non-invasive measurement tools is expected.

By the emersion of MDCT, the imaging diagnosis of portal hemodynamics, which contains esophageal gastric varices and other collateral veins, has progressed and has been applied to the decision of therapeutic strategies and the evaluation of the treatment effect.[9,10]

Endoscopic examination is an established method to evaluate esophageal varices. It is performed at periodic intervals based on guidelines to detect esophageal varices in patients with cirrhosis, but it lays a burden on patients physically and economically. In addition, esophageal varices shows a variable prevalence, ranging from 0–10% in patients with compensated disease, to 70–80% in patients with decompensated cirrhosis (mean, 40–55%) at diagnosis.[11] For these reasons, selection of patients with a high probability of bearing esophageal varices and large esophageal varices at risk of rupture has been proposed using various non-invasive methods, such as FibroTest[12] or spleen/platelet count ratio,[13] transient elastography.[5–8]

The liver stiffness measurement by transient elastography (FibroScan) is performed to estimate the staging of liver fibrosis. This measurement procedure could be performed repeatedly because of its non-invasiveness. Several groups reported that liver stiffness as measured by FibroScan correlates to portal vein pressure and useful in the prediction of presence of esophageal varices.

Kazemi *et al.* examined the relationship between esophageal varices and liver stiffness in patients with chronic liver injury caused by various etiological factors and documented that liver stiffness correlated to grade of esophageal varices, and that liver stiffness ≤ 19 kPa indicated a low probability of esophageal varices.[7] Carrion *et al.* showed that liver stiffness of patients with recurrent hepatitis C following liver transplantation correlated to hepatic vein pressure gradient (HVPG) and severity of liver fibrosis as assessed by transvenous liver biopsy, suggesting that liver stiffness might be useful in assessing recurrent hepatitis C following liver transplantation and reducing the number of liver biopsies.[5]

Thus, by the progress of the assessment tools, the clinical condition of portal hypertension has been grasped more precisely and more easily than before. Moreover, the non-invasive assessment tool may be useful in the repeatable assessment of liver function, staging, and prediction of prognosis in the natural history of portal hypertension.

TREATMENT OF PORTAL HYPERTENSION BY INTERVENTIONAL RADIOLOGY (IVR) PROCEDURE

BALLOON OCCLUDED RETROGRADE transvenous obliteration (B-RTO) is an IVR procedure developed for the treatment of fundic varices[14] and has been increasingly accepted in Asian countries. Its technique is performed by the occlusion of gastro-renal shunt (GR shunt) by inflating a balloon catheter and injection of 5% ethanolamine oleate iopamidol (EOI) for overnight. The eradiation rate of fundic varices is high (87–100%) and recurrence rate is extremely low (0–10%).[14–17] B-RTO also decreases a blood ammonia level and improves shunt derived encephalopathy. A possible adverse effect is the appearance of ascites and esophageal varices by the elevation of portal vein pressure. Worsening rate of esophageal varices is 19–62.5%.[14–17] B-RTO increases a portal venous flow and portal pressure. Several groups reported that increased serum albumin levels and improvement of Child-Pugh score are observed after B-RTO.[15–17] As to a long-term result, the reported survival rate at 1, 3, and 5 years were 83.1–97.6%, 69–76%, and 54–68%, respectively.[17–22] In Japan, the B-RTO procedure has been becoming the major prophylactic treatment for fundic varices.

Partial splenic embolization (PSE) has been increasingly performed as a non-surgical treatment by IVR procedure for the treatment of hematological disorders caused by hypersplenism, such as leucopenia and thrombocytopenia.[23,24] Moreover, PSE is reported to be effective in improving liver function,[25] reducing portal pressure and decrease the episodes of variceal bleeding by combined therapy with endoscopic variceal ligation (EVL),[26] and decreasing the bleeding episode of portal hypertensive gastropathy (PHG).[27] Although a thrombocytopenia excludes the use of pegylated interferon and ribavirin in cirrhotic patients with hepatitis C virus infection, PSE allows the safe use of pegylated interferon plus ribavirin in cirrhotic patients with hepatitis C viral infection.[28] Hepatocellular carcinoma (HCC) is often associated with a bleeding tendency due to hypersplenism and a high incidence of hemorrhagic complication due to treatment and chemotherapy induces lowering of peripheral blood cell counts by bone marrow suppression. PSE combined with transcatheter hepatic arterial chemoembolization (TACE) can improve the tolerance of HCC patients with cirrhosis and hypersplenism and reduce the risk of complication.[29]

Thus, PSE is a promising and safe procedure, not only to control hypersplenism but also to improve their liver

function and prognosis by expanding the treatment indication, which had been excluded from the treatment due to low platelet count.

PHARMACOLOGICAL TREATMENT OF PORTAL HYPERTENSION

ANGIOTENSIN II (AT-II) is a potential mediator of intrahepatic portal hypertension. In the cirrhosis patient, the rennin angiotensin system (RAS) is frequently activated, and plasma level of AT-II is elevated. AT-II induces sodium and fluid retention by the stimulation of aldosterone secretion. In addition, AG II induces contractile influence on hepatic stellate cells (HSC), which serve as regulators of the sinusoidal blood flow and increase in intrahepatic vascular resistance.[30]

Angiotensin II receptor type 1 blockers (ARBs), including Losartan,[31] Irbesartan,[32] Candesartan,[33] Olmesartan,[34] have been reported to decrease the portal vein pressure. Schneider firstly studied the effect of Losartan in patients with portal hypertension and demonstrated a significant portal hypotensive response in excess of 40%, accomplished with minimal effects on the systemic circulation. However, recent study failed to confirm the findings of Schneider in a large group of cirrhotic patients.[35] Therefore, there remains uncertainty about the efficacy.

On the other hands, AT-II has been suggested to play an important role in liver fibrogenesis. It induces HSC proliferation and up-regulates the transforming growth factor beta1 expression via AT-II type 1 receptor. ARBs have a potent antifibrotic effect.[36] Termisartan prevents liver fibrogenesis by inhibiting the HSCs activation and proliferation, in the mechanism of down-regulating TGF beta1 and TIMP-1, 2 and increasing MMP-13 expression.[37] Thus, the ARBs are promising drugs for portal hypertension with both portal hypotensive effect and antifibrotic effects.

ANTI-FIBROTIC THERAPY FOR PORTAL HYPERTENSION

RECENTLY, IT HAS been recognized that liver fibrosis is reversible if the hepatic inflammation is completely removed. Shiratori performed the liver biopsy in hepatitis C virus (HCV)-related patients before and after the interferon therapy and showed that the rate of fibrosis progression, calculated as the change in fibrosis stage per year, was approximately 0.1 unit/ year in untreated patients. Conversely, the liver fibrosis was found to have regressed in patients with sustained response at a rate of −0.28 unit/ year.[38] Rincon showed that antiviral therapy significantly reduces portal pressure in patients with compensated METAVIR stage F3 and F4 chronic hepatitis C and portal hypertension. Twenty compensated patients received PEG-Interferon α2b plus ribavirin therapy and HVPG significantly dropped in all but one treated patient, with a mean reduction of 28.2%. The percentage of HVPG decrease was significantly greater in patients who achieved a virological end of treatment response.[39] These results show that complete disappearance of the inflammation by the eradiation therapy for the hepatitis virus induces the reversibility of liver fibrosis and has the possibility to reverse the portal hypertension.

The plasticity of bone marrow cells (BMC) has been confirmed by autopsy results of female recipients of BMC from male donors.[40,41] To establish new clinical therapies for patients with liver cirrhosis using autologous BMC, we developed a new *in vivo* murine model using green fluorescent protein (GFP) and repeated carbon tetrachloride (CCl4) injection.[42] We found that BMC infused through the tail vein, efficiently repopulated cirrhotic liver tissue and, under the influence of persistent liver damage induced by carbon tetrachloride, differentiated into albumin-producing hepatocytes. Moreover, such BMC infusions into mice with cirrhosis improved liver function and reduced mortality. The latter observation correlated with the strong expression of matrix metalloproteinases (MMP), particularly MMP-9, and reduced hepatic fibrosis.[43]

We have performed a clinical study of 23 cases of cirrhosis treated by autologous BMC infusion (ABMi) via a peripheral vein. Significant improvements in serum albumin levels and total protein were seen at 24 weeks after ABMi therapy ($P < 0.05$), while the Child-Pugh score improved significantly at 4 and 24 weeks ($P < 0.05$). The serum concentration of the fibrotic marker, N-terminal procollagen III peptide (P-III-P) tended toward improvement.[44] In one case, an increase of portal blood flow and decrease of diameter of portal vein at 24 weeks is observed and the suggested that ABMi therapy may reduce portal hypertension.

From these results, antifibrotic therapy may have the possibility of decreasing a resistance of portal venous pressure and improve portal hypertension. Further investigation is necessary.

CONCLUSION

1 Assessment of liver stiffness may predict the existence of esophageal varices.

2 BRTO procedure will have a possibility to improve not only portal hemodynamics but also prognosis.
3 IFN therapy combined with PSE may reverse liver fibrosis resulting in improvement of portal hypertension.
4 ARBs may be beneficial for the treatment of portal hypertension, but further long-term studies are necessary.
5 Anti-fibrotic therapy, e.g. ABM*i* therapy may have the possibility of reducing portal hypertension.

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Genome-wide association of *IL28B* with response to pegylated interferon-α and ribavirin therapy for chronic hepatitis C

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The recommended treatment for patients with chronic hepatitis C, pegylated interferon-α (PEG-IFN-α) plus ribavirin (RBV), does not provide sustained virologic response (SVR) in all patients. We report a genome-wide association study (GWAS) to null virological response (NVR) in the treatment of patients with hepatitis C virus (HCV) genotype 1 within a Japanese population. We found two SNPs near the gene *IL28B* on chromosome 19 to be strongly associated with NVR (rs12980275, $P = 1.93 \times 10^{-13}$, and rs8099917, 3.11×10^{-15}). We replicated these associations in an independent cohort (combined *P* values, 2.84×10^{-27} (OR = 17.7; 95% CI = 10.0–31.3) and 2.68×10^{-32} (OR = 27.1; 95% CI = 14.6–50.3), respectively). Compared to NVR, these SNPs were also associated with SVR (rs12980275, $P = 3.99 \times 10^{-24}$, and rs8099917, $P = 1.11 \times 10^{-27}$). In further fine mapping of the region, seven SNPs (rs8105790, rs11881222, rs8103142, rs28416813, rs4803219, rs8099917 and rs7248668) located in the *IL28B* region showed the most significant associations ($P = 5.52 \times 10^{-28}$–2.68×10^{-32}; OR = 22.3–27.1). Real-time quantitative PCR assays in peripheral blood mononuclear cells showed lower *IL28B* expression levels in individuals carrying the minor alleles ($P = 0.015$).

Hepatitis C is a global health problem that affects a significant proportion of the world's population. The World Health Organization estimated that in 1999, there were 170 million HCV carriers worldwide, with 3–4 million new cases appearing each year. HCV infection affects more than 4 million people in the United States, where it represents the leading cause of cirrhosis and hepatocellular carcinoma as well as the leading cause of liver transplantation[1].The American Gastroenterological Association estimated that drugs are the largest direct costs of hepatitis C[1].

The most effective current standard of care in patients with chronic hepatitis C, a combination of PEG-IFN-α with ribavirin, does not produce SVR in all patients treated. Large-scale studies on 48-week-long PEG-IFN-α/RBV treatment in the United States and Europe showed that 42–52% of patients with HCV genotype 1 achieved SVR[2–4], and similar results were found in Japan. However, older patients (greater than 50 years of age) had a significantly lower rate of SVR due to poor adherence resulting from adverse events and laboratory-detectable abnormalities such as neutropenia and thrombocytopenia[5,6]. Specifically, various well-described side effects (such as a flu-like syndrome, hematologic abnormalities and adverse neuropsychiatric events) often necessitate dose reduction, and 10–14% of patients require premature withdrawal from interferon-based therapy[7]. To avoid these side effects in patients who will not be helped by the treatment, as well as to reduce the substantial cost of PEG-IFN-α/RBV treatment, it would be useful to be able to predict an individual's response before or early in treatment. Several viral factors, such as genotype 1, high baseline viral load, viral

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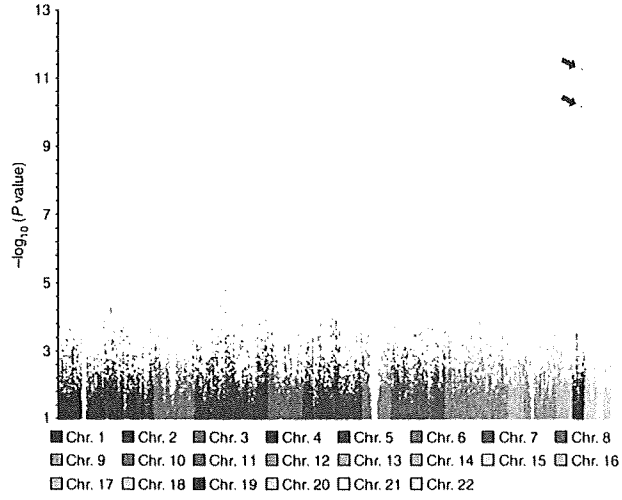

Figure 1 Genome-wide association results with PEG-IFN-α/RBV treatment in 142 Japanese patients with HCV (78 NVR and 64 VR samples). *P* values were calculated by using a χ^2 test for allele frequencies. The dots with arrows for chromosome 19 denote SNPs that showed significant genome-wide associations ($P < 8.05 \times 10^{-8}$) with response to PEG-IFN-α/RBV treatment.

kinetics during treatment, and amino acid pattern in the interferon sensitivity–determining region, have been reported to be significantly associated with the treatment outcome in a number of independent studies[8–10]. Studies have also provided strong evidence that ~20% of patients with HCV genotype 1 and 5% of patients with genotype 2 or 3 have a null response to PEG-IFN-α/RBV. No definite predictor of this resistance is currently available that make it possible to bypass the initial 12–24 weeks' treatment before deciding whether treatment should be continued. If a reliable predictor of non-response were identified for use in patients before treatment initiation, then an estimated 20%, including those who have little or no chance to achieve SVR, could be spared the side effects and cost of treatment.

Host factors, including age, sex, race, liver fibrosis and obesity, have also been reported to be associated with PEG-IFN-α/RBV therapy outcome[11,12]. However, little is known about the host genetic factors that might be associated with the response to therapy: thus far only a few candidate genes, including those encoding type I interferon receptor-1 (*IFNAR1*) and mitogen-activated protein kinase–activated protein kinase 3 (*MAPKAPK3*), have been reported to be associated with treatment response[13,14]. We describe here a GWAS for response to PEG-IFN-α/RBV treatment.

We conducted this GWAS to identify host genes associated with response to PEG-IFN-α/RBV treatment in 154 Japanese patients with HCV genotype 1 (82 with NVR and 72 with virologic response (VR), based on the selection criteria as described in Online Methods). We used the Affymetrix SNP 6.0 genome-wide SNP typing array for 900,000 SNPs. A total of 621,220 SNPs met the following criteria: (i) SNP call rate ≥95%, (ii) minor allele frequency (MAF) ≥1% and (iii) deviation from Hardy-Weinberg equilibrium (HWE) $P \geq 0.001$ in VR samples. After excluding 4 NVR and 8 VR samples that showed quality control (QC) call rates of <95%, 78 NVR and 64 VR samples were included in the association analysis. **Figure 1** shows a genome-wide view of the single-point association data based on allele frequencies. Two SNPs located close to *IL28B* on chromosome 19 showed strong associations, with a minor allele dominant model (rs12980275, $P = 1.93 \times 10^{-13}$, and rs8099917, $P = 3.11 \times 10^{-15}$, respectively), with NVR to PEG-IFN-α/RBV treatment (**Table 1**). The rs8099917 lies between *IL28B* and *IL28A*, ~8 kb downstream from *IL28B* and ~16 kb upstream from *IL28A*. These associations reached genome-wide levels of significance for both SNPs in this initial GWAS cohort (Bonferroni criterion $P < 8.05 \times 10^{-8}$ (0.05/621,220)). The frequencies of minor allele–positive patients were much higher in the NVR group than in the VR group for both SNPs (74.3% in NVR, 12.5% in VR for rs12980275; 75.6% in NVR, 9.4% in VR for rs8099917). Notably, individuals homozygous for the minor allele were observed only in the NVR group. The VR group, as compared to the NVR group, showed genotype frequencies closer to those in the healthy Japanese population[15], yet the minor allele frequencies were slightly higher in the transient virologic response (TVR) group (23.1%, 15.4%) than in the SVR group (9.8%, 7.8%) (**Table 1**). We applied the Cochrane-Armitage test on all the SNPs and found a genetic inflation factor, λ, of 1.029 for the GWAS stage (**Supplementary Fig. 1**). We also carried out principal component analysis in 142 samples for the GWAS stage together with the HapMap samples (CEU, YRI, CHB and JPT) (**Supplementary Fig. 2**); this suggested that the effect of population stratification was negligible.

Table 1 Significant association of two SNPs (rs12980275 and rs8099917) with response to PEG-IFN-α/RBV treatment

					Null responder (NVR[a], *n* = 128)			Responder (VR[a], *n* = 186)			Responder (SVR[a], *n* = 140)			NVR vs. VR		NVR vs. SVR	
dbSNP rsID	Nearest gene	MAF[b] (allele)	Allele (1/2)	Stage	11	12	22	11	12	22	11	12	22	OR (95% CI)[c]	*P* value[d]	OR (95% CI)[c]	*P* value[d]
rs12980275	*IL28B*	0.15 (G)	A/G	GWAS	20 (25.6)	54 (69.2)	4 (5.1)	56 (87.5)	8 (12.5)	0 (0.0)	46 (90.2)	5 (9.8)	0 (0.0)	20.3 (8.3–49.9)	1.93×10^{-13}	26.7 (9.3–76.5)	7.41×10^{-13}
				Replication	10 (20.0)	37 (74.0)	3 (6.0)	101 (82.8)	21 (17.2)	0 (0.0)	73 (82.0)	16 (18.0)	0 (0.0)	19.2 (8.3–44.4)	5.46×10^{-15}	18.3 (7.6–44.0)	8.37×10^{-13}
				Combined	30 (23.4)	91 (71.1)	7 (5.5)	157 (84.4)	29 (15.6)	0 (0.0)	119 (85.0)	21 (15.0)	0 (0.0)	17.7 (10.0–31.3)	2.84×10^{-27}	18.5 (10.0–34.4)	3.99×10^{-24}
rs8099917	*IL28B*	0.12 (G)	T/G	GWAS	19 (24.4)	56 (71.8)	3 (3.8)	58 (90.6)	6 (9.4)	0 (0.0)	47 (92.2)	4 (7.8)	0 (0.0)	30.0 (11.2–80.5)	3.11×10^{-15}	36.5 (11.6–114.6)	5.00×10^{-14}
				Replication	11 (22.0)	37 (74.0)	2 (4.0)	108 (88.5)	14 (11.5)	0 (0.0)	78 (87.6)	11 (12.4)	0 (0.0)	27.4 (11.5–65.3)	9.47×10^{-18}	25.1 (10.0–63.1)	1.00×10^{-14}
				Combined	30 (23.4)	93 (72.7)	5 (3.9)	166 (89.2)	20 (10.8)	0 (0.0)	125 (89.3)	15 (10.7)	0 (0.0)	27.1 (14.6–50.3)	2.68×10^{-32}	27.2 (13.9–53.4)	1.11×10^{-27}

[a]NVR, null virologic response; VR, virologic response; SVR, sustained virologic response. The 186 VRs consisted of 46 transient virologic response (TVRs) and 140 SVRs. [b]Minor allele frequency and minor allele in 184 healthy Japanese individuals[15]. The MAF of the SNPs in SVR is similar to that of TVR group, whereas that of NVR is much higher (76.6%). [c]Odds ratio for the minor allele in a dominant model. [d]*P* value by χ^2 test for the minor allele dominant model.

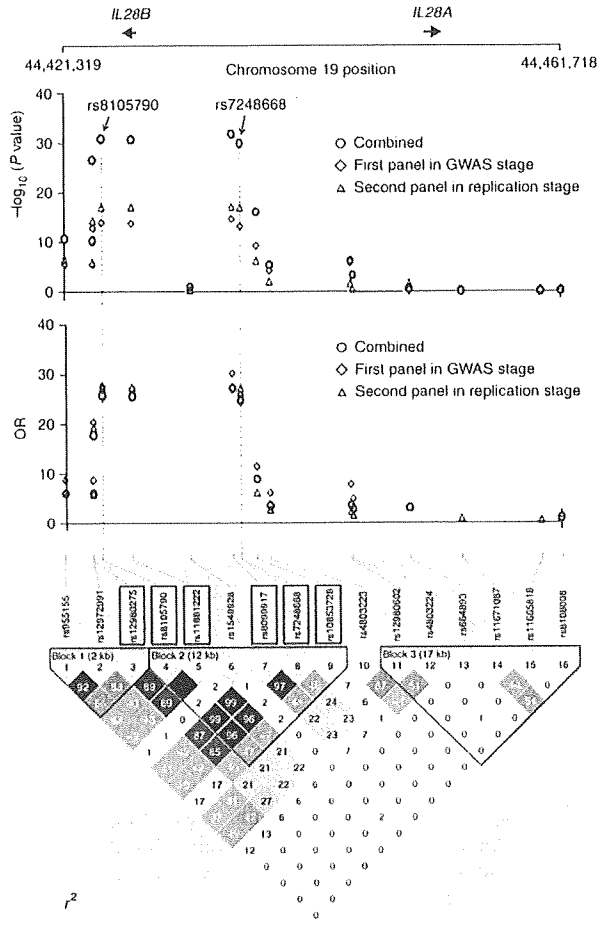

Figure 2 Genomic structure, *P* value and OR plots in association analysis and LD map around *IL28B* and *IL28A* (chr.19, nucleotide positions 44421319–44461718; build 35). *P* values by the χ^2 test for minor allele dominant effect model are shown for the first panel of 142 samples in the GWAS stage, the second panel of 172 samples in the replication stage, and the combined analysis. Below are estimates of pairwise r^2 for 16 SNPs selected in the replication study using a total of 314 Japanese patients with HCV treated with PEG-IFN-α/RBV. Boxes indicate the significantly associated SNPs with response to PEG-IFN-α/RBV treatment both in the GWAS stage and in the replication stage. Dotted lines indicate the region with the strongest associations from the positions of rs8105790 to rs7248668.

We analyzed the region of ~40 kb (chr. 19, nucleotide positions 44421319–44461718; build 35) containing the significantly associated SNPs (rs12980275 and rs8099917) using Haploview software for linkage disequilibrium (LD) and haplotype structure based on the HapMap data for individuals of Japanese ancestry. The LD blocks were analyzed using the four-gamete rule, and four blocks were observed (**Supplementary Fig. 3**). We selected 16 SNPs for both replication study and high-density association mapping, including tagging SNPs estimated on the basis of the haplotype blocks, one SNP located within *IL28B* (rs11881222) and the significantly associated SNPs from the GWAS stage (rs12980275 and rs8099917) (**Supplementary Table 1**).

To validate the results of the GWAS stage, 16 SNPs selected for the replication stage, including the original SNPs, were genotyped using the DigiTag2 assay in an independent set of 172 Japanese patients with HCV treated with PEG-IFN-α/RBV treatment (50 NVR and 122 VR samples), together with the first panel of 142 samples analyzed in the GWAS stage (**Supplementary Table 1**). The associations of the original SNPs were replicated in the replication cohort of 172 patients ($P = 5.46 \times 10^{-15}$, OR = 19.2 for rs12980275; $P = 9.47 \times 10^{-18}$, OR = 27.4 for rs8099917; **Table 1**). The combined *P* values for both stages reached 2.84×10^{-27} (OR = 17.7; 95% CI = 10.0–31.3) and 2.68×10^{-32} (OR = 27.1; 95% CI = 14.6–50.3), respectively (**Table 1**). Notably, when we compared the SVR ($n = 140$) with the NVR group ($n = 128$), the original two SNPs (rs12980275 and rs8099917) again showed strong associations: both *P* values and ORs were similar to those observed in the comparison between VR and NVR, and the combined *P* values for both stages reached 3.99×10^{-24} (OR = 18.5; 95% CI = 10.0–34.4) and 1.11×10^{-27} (OR = 27.2; 95% CI = 13.9–53.4), respectively (**Table 1**). Comparing SVR ($n = 140$) versus NVR plus TVR ($n = 174$), we again found that these SNPs were significantly associated ($P = 1.71 \times 10^{-16}$, OR = 8.8; 95% CI 5.1–15.4 for rs12980275; $P = 1.18 \times 10^{-18}$, OR = 12.1; 95% CI 6.5–22.4 for rs8099917, **Supplementary Table 2**), suggesting that these SNPs would predict NVR as well as SVR before PEG-IFN-α/RBV therapy.

Among the newly analyzed SNPs in the replication study, six (rs12980275, rs8105790, rs11881222, rs8099917, rs7248668 and rs10853728) showed significant associations both in the GWAS stage ($P < 8.05 \times 10^{-8}$) and in the replication stage ($P < 0.0031$ (0.05/16)) after Bonferroni correction. These SNPs are located within a 15.7-kb region that includes *IL28B* (**Fig. 2** and **Supplementary Table 1**). In particular, the strongest associations with NVR were observed for four SNPs, rs8105790, rs11881222, rs8099917 and rs7248668, that are located in the downstream flanking region, the third intron and the upstream flanking region of *IL28B*. The combined *P* values for these polymorphisms were 1.98×10^{-31} (OR = 25.7; 95% CI = 13.9–47.6), 2.84×10^{-31} (OR = 25.6; 95% CI = 13.8–47.3), 2.68×10^{-32} (OR = 27.1; 95% CI = 14.6–50.3) and 1.84×10^{-30} (OR = 24.7; 95% CI = 13.3–45.8), respectively (**Supplementary Table 1**). We then sequenced this region to identify further variants and found three SNPs (rs8103142, rs28416813 and rs4803219) located in the third exon, the first intron and the upstream flanking region of *IL28B*, and a few infrequent variations. These SNPs also showed strong associations in the combined dataset of 128 NVR and 186 VR samples ($P = 1.40 \times 10^{-29}$, OR = 26.6 for rs8103142; $P = 5.52 \times 10^{-28}$, OR = 22.3 for rs28416813; $P = 2.45 \times 10^{-29}$, OR = 23.3 for rs4803219; **Supplementary Table 3**). We also performed LD and haplotype analyses with seven SNPs. These SNPs were in strong LD, and the risk haplotype showed a level of association similar to those of individual SNPs ($P = 1.35 \times 10^{-25}$, OR = 11.1; 95% CI = 6.6–18.6) (**Table 2**). These results suggest that the association with NVR was primarily driven by one of these SNPs.

Table 2 Association analysis of response to treatment by *IL28B* haplotype

SNP							Frequencies			
rs8105790	rs11881222	rs8103142	rs28416813	rs4803219	rs8099917	rs7248668	NVR group	VR group	*P* value	OR (95% CI)
T	A	T	C	C	T	G	0.543	0.942	1.81×10^{-32}	0.1 (0.04–0.12)
C	G	C	G	T	G	A	0.387	0.054	1.35×10^{-25}	11.1 (6.6–18.6)

Association analysis of haplotypes consisting of seven SNPs with response to PEG-IFN-α/RBV treatment in 314 Japanese patients with HCV. Boldface letters: rs11881222 (third intron); rs8103142 (third exon).

Table 3 Factors associated with NVR by logistic regression model

Factors	Odds ratio	95% CI	*P* value
rs8099917 (G allele)	37.68	16.71–83.85	<0.0001
Age	1.02	0.98–1.07	0.292
Gender (Female)	3.32	1.49–7.39	0.003
Re-treatment[a]	1.12	0.55–2.33	0.750
Platelet count	0.93	0.87–1.01	0.080
Aminotransferase level	1.00	0.99–1.00	0.735
Fibrosis stage[20]	1.10	0.73–1.66	0.658
HCV-RNA level	1.01	0.99–1.02	0.139

[a]Re-treatment, non-response to previous treatment with interferon-α (plus RBV).

To examine the relative contribution of factors associated with NVR, we used a logistic regression model. One tagging SNP located within *IL28B* (minor allele of rs8099917) was the most significant factor for predicting NVR, followed by gender (**Table 3**). Clinically, viral factors such as HCV genotype and HCV RNA level are important for the outcome of PEG-IFN-α/RBV therapy. Indeed, mean HCV-RNA level was significantly lower in SVR (SVR versus TVR, $P = 0.002$; SVR versus NVR, $P = 0.016$; **Supplementary Table 4**). Mean platelet count and the proportion of mild fibrosis (F1–F2) were significantly higher in SVR than in NVR.

Real-time quantitative PCR assays in peripheral blood mononuclear cells revealed a significantly lower level of *IL28* mRNA expression in individuals with the minor alleles (**Fig. 3**), suggesting that variant(s) regulating *IL28* expression is associated with a response to PEG-IFN-α/RBV treatment. *IL28B* encodes a cytokine distantly related to type I (α and β) interferons and the interleukin (IL)-10 family. This gene and *IL28A* and *IL29* (encoding IL-28A and IL-29, respectively) are three closely related cytokine genes that encode proteins known as type III IFNs (IFN-λs) and that form a cytokine gene cluster at chromosomal region 19q13 (ref. 16). The three cytokines are induced by viral infection and have antiviral activity[16,17]. All three interact with a heterodimeric class II cytokine receptor that consists of IL-10 receptor beta (IL10Rβ) and IL-28 receptor alpha (IL28Rα, encoded by *IL28RA*)[16,17], and they may serve as an alternative to type I IFNs in providing immunity to viral infection.

Notably, a recent report showed that the strong antiviral activity evoked by treating mice with TLR3 or TLR9 agonists was significantly reduced in both $IL28RA^{-/-}$ and $IFNAR^{-/-}$ mice, indicating that IFN-λ is important in mediating antiviral protection by ligands for TLR3 and TRL9 (ref. 18). IFN-λ induced a steady increase in the expression of a subset of IFN-stimulated genes, whereas IFN-α induced the same genes with more rapid and transient kinetics[19]. Therefore, it is possible that IFN-λ induces a slower but more sustained response that is important for TLR-mediated antiviral protection. This might be one of the ways that a genetic variant regulating *IL28* expression influences the response to PEG-IFN-α/RBV treatment. Further research will be required to fully understand the specific mechanism by which a genotype might affect the response to treatment.

In conclusion, the strongest associations with NVR were observed for seven SNPs, rs8105790, rs11881222, rs8103142, rs28416813, rs4803219, rs8099917 and rs7248668, that are located in the downstream flanking region, the third intron, the third exon, the first intron and the upstream flanking region of *IL28B*. Further studies following our report of this robust genetic association to NVR may make it possible to develop a pre-treatment predictor of which individuals are likely to respond to PEG-IFN-α/RBV treatment. This would remove the need for the initial 12–24 weeks of treatment that is currently used as a basis for a clinical decision about whether treatment should be continued. That would allow better targeting of PEG-IFN-α/RBV treatment, avoiding the unpleasant side effects that commonly accompany the treatment where it is unlikely to be beneficial, and reduce overall treatment costs. Because of the small number of samples in this study, we plan to conduct a further prospective multicenter study to establish these SNPs as a clinically useful marker.

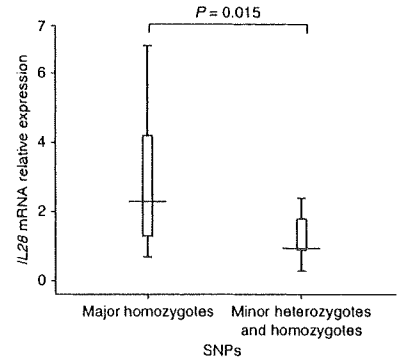

Figure 3 Quantification of *IL28* mRNA expression. The expression level of *IL28* genes was determined by real-time quantitative RT-PCR using RNA purified from peripheral blood mononuclear cells. Distribution of relative gene expression levels was compared between the individuals homozygous for major alleles ($n = 10$) and the heterozygous or homozygous individuals carrying minor alleles ($n = 10$) of rs8099917 by using the Mann-Whitney *U*-test. The bars indicate the median. All samples were obtained from HCV-infected patients before PEG-IFN-α/RBV therapy.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

Study design and discussion: Y.T., N.N., N.M., K.T., M.M.; sample collection: Y.T., M.K., K.M., N.S., M.N., M.K., K.H., S.H., Y.I., E.M., E.T., S.M., Y.M., M.H., A.S., Y.H., S.N., I.S., M.I., K.I., K.Y., F.S., N.I.; genotyping: N.N.; statistical analysis: N.N., A.K., K.I.; quantitative RT-PCR: M.S.; manuscript writing: Y.T., N.N., K.T., M.M.

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Use of the light-emitting diode-illuminated endoscope for upper gastrointestinal endoscopy

We have developed the white light-emitting diode (LED)-illuminated endoscopes and previously reported our preliminary experiments with the prototype LED endoscope in the beagle [1]. Authors in *Nature* noted that the white LEDs we employed have been used for illuminating paintings in fine art museums, as they emit white light with good color rendering and homogeneous light distribution [2,3]. This supports the notion that these LEDs may also be suitable for use in gastrointestinal endoscopes, as these instruments must be able to indicate subtle changes in the color and mucosal structure of the gastrointestinal tract.

The prototype LED endoscopes were based on conventional endoscopes for studies in humans (Fujifilm Corporation, Saitama, Japan), and white LEDs were mounted on their tip (◎ **Fig. 1**).

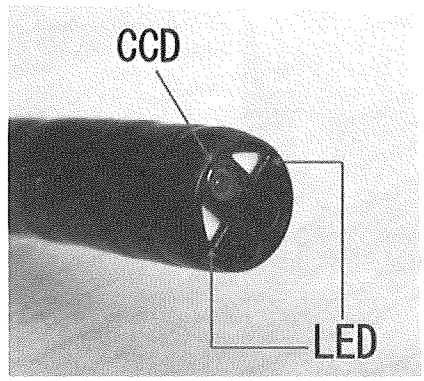

Fig. 1 The prototype light-emitting diode (LED) endoscope. Two packages of white LEDs and a charge-coupled device (CCD) were attached to the distal end of the prototype LED endoscope.

After obtaining Institutional Review Board approval, a patient with an early gastric carcinoma underwent endoscopy using this new endoscope.

We found that the white LEDs did not provide sufficient illumination for distant observation in the stomach. However, our prototype LED endoscope allowed clear visualization of the early gastric cancer by close observation. The LED endoscope showed a flat lesion, which was located at the anterior wall of the mid-gastric body and had ill-defined margins accompanied by slightly reddish or focally pale mucosa (◎ **Fig. 2a**).

Indigo carmine chromoendoscopy emphasized the redness of the lesion and delineated the demarcation between the cancerous and non-cancerous mucosa (◎ **Fig. 2b**). These findings corresponded to those from an examination with a conventional endoscope (◎ **Fig. 2c,d**). We then subjected the lesion to endoscopic submucosal dissection using a conventional endoscope. Pathological evaluation of the excised tissue revealed that the lesion was an intramucosal differentiated adenocarcinoma and that both its lateral and vertical margins were free of carcinoma cells (◎ **Fig. 3a,b**).

In conclusion, our observations show that our prototype LED-illuminated endoscope can clearly visualize early gastric cancers upon close observation.

Endoscopy_UCTN_Code_TTT_1AO_2AN

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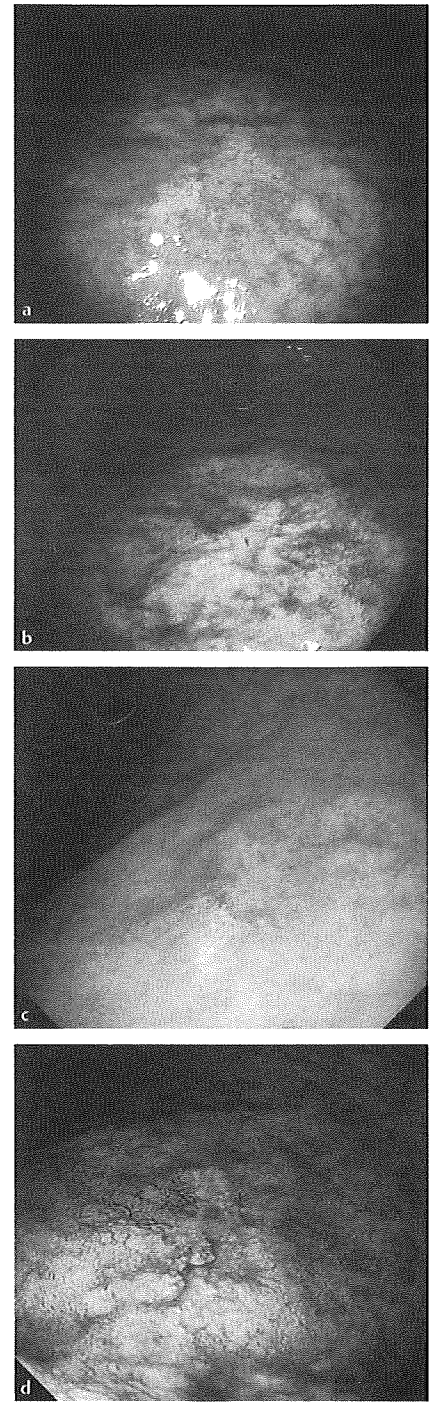

Fig. 2 An image of a superficially depressed-type early gastric carcinoma that was obtained using the LED endoscope and a conventional endoscope: **a** White LED endoscopic view; **b** view with indigo carmine dye. **c** Conventional endoscopic view; **d** view with indigo carmine dye.

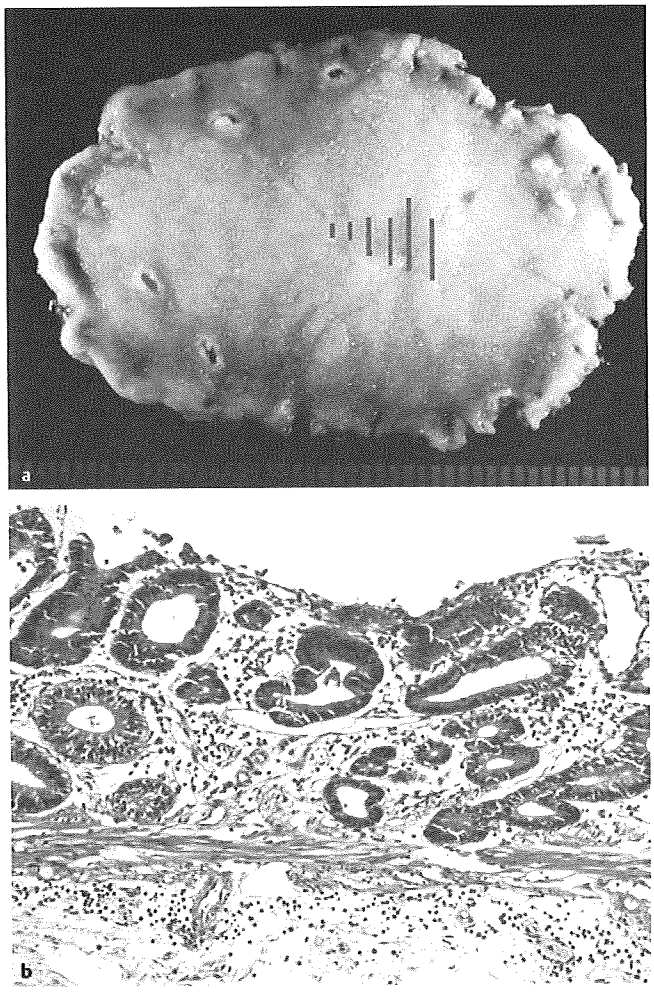

Fig. 3 **a** Macroscopic view of the resected specimen. The red lines indicate the carcinoma area. **b** Pathological analysis of the resected tissue revealed a well-differentiated adenocarcinoma in the mucosal layer (× 200).

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ERCP using double-balloon endoscopes in patients with Roux-en-Y anastomosis

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Abstract
Introduction Endoscopic retrograde cholangiopancreatography (ERCP) and associated procedures are difficult to perform in patients with a Roux-en-Y reconstruction. Therefore, at present, at many institutions, ERCP is not generally performed for those with a Roux-en-Y anastomosis.
Methods However, double-balloon endoscopes (DBEs) have dramatically changed this situation.
Results The use of a DBE enables an endoscopic approach into the deeply situated small intestine, which has been difficult with a conventional endoscope. Therefore, ERCP for patients with a Roux-en-Y anastomosis has been attempted using a DBE, and good results have been reported.
Conclusion The development of DBEs has created the possibility of performing ERCP for patients with Roux-en-Y reconstruction in whom an endoscopic approach has conventionally been believed to be difficult.

Keywords Double-balloon endoscope · Double-balloon endoscopy · ERCP · Roux-en-Y

Introduction

Endoscopic retrograde cholangiopancreatography (ERCP) and associated procedures are difficult to perform in patients with a Roux-en-Y reconstruction. In the patient with a Roux-en-Y reconstruction, the long afferent loop can lead to looping of the endoscope, and strong adhesions can make it technically more difficult to reach the papilla of Vater or the bilio-enteric anastomosis. In the past, in patients with a Roux-en-Y anastomosis, conventional side-viewing endoscopes [1, 2], pediatric colonoscopes, [3, 4] and a forward-viewing endoscope with the use of an overtube [5] have been used for ERCP, but success has been achieved mainly due to the skill and determination of the operator, so ERCP for these patients has been regarded as an unrealistic procedure at many institutions. However, double-balloon endoscopes (DBEs) [6, 7] have dramatically changed the situation. The use of a DBE allows for an endoscopic approach into the deeply situated small intestine, which has been difficult with a conventional endoscope. Thus, ERCP for patients with a Roux-en-Y anastomosis has been attempted using DBEs, and good results have been reported [8–17]. In this review, the actual situation of ERCP in which a DBE is used for patients with a Roux-en-Y anastomosis is described in general, with regard to its procedures, results, and problems.

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Types of double-balloon endoscopes (DBEs)

Table 1 lists the specifications of the three types of DBEs (Fujinon Toshiba ES Systems, Tokyo, Japan) that are currently commercially available in Japan. The EN-450P5/20 is a diagnostic endoscope, and because the diameter of the working channel is small, at 2.2 mm, accessories for ERCP cannot be inserted thereinto. Haruta et al. [8] who reported on ERCP using the EN-450P5/20 DBE for the first time, performed cholangiography using a 4-French catheter. Subsequently, they removed the DBE, leaving the overtube and the guide wire, and then expanded the anastomosed region using a 6-French dilation catheter under fluoroscopic control. Emmett et al. [11] also performed ERCP using the EN-450P5/20 in order to perform stone removal, bile-duct

Table 1 Specifications of double-balloon endoscopes

Endoscope	EN-450P5/20	EN-450T5/W	EC-450BI5
Diameter (mm)	8.5	9.4	9.4
Working length (mm)	2000	2000	1520
Working channel (mm)	2.2	2.8	2.8
Overtube	TS-12140	TS-13140	TS-13101
Diameter (mm)	12.2	13.2	13.2
Length (mm)	1450	1450	1050

Fujinon Toshiba ES Systems, Tokyo, Japan

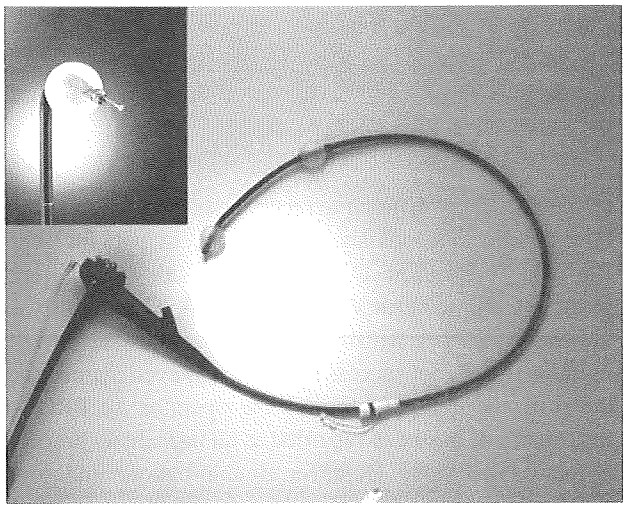

Fig. 1 "Short" double-balloon endoscope (EC-450BI5; Fujinon Toshiba ES Systems, Tokyo, Japan) with a working length of 1520 mm and a working channel of 2.8 mm

dilation, sphincterotomy, stent placement, and the like, but it is not known which treatment tools were used.

The EN-450T5/W is a therapeutic endoscope having a working-channel diameter of 2.8 mm, and some accessories for ERCP can be inserted thereinto. This DBE has been used as stated in many previous reports [10, 12–14, 16]. However, the working length is so long, at 2000 mm, that the accessories that can be used for ERCP are limited. This is because many accessories for ERCP are shorter than 2000 mm.

The EC-450BI5 (Fig. 1) is a "short" double-balloon endoscope that was developed in 2006 [15] and has a large working-channel diameter of 2.8 mm and a short working length of 1520 mm, so many commercially available accessories for ERCP can be used.

Problems and innovations in using double-balloon endoscopes (DBEs)

In the previous reports of ERCP using DBEs, the EN-450T5/W, with a working length of 2000 mm was the most frequently used type [10, 12–14, 16]. This is because many institutions have this type of DBE for the diagnosis and treatment of small-intestinal disorders. However, in recent years, shorter accessories for ERCP, rather than longer ones, have tended to be developed, for convenience [18]. Therefore, when the EN-450T5/W is used, the accessories that can be used for ERCP are limited, thereby causing inconvenience, because the working length is long, at 2000 mm. An innovation for overcoming this inconvenient situation is that, after the DBE has reached the papilla of Vater or the bilio-enteric anastomosis, the DBE is replaced with a conventional upper gastrointestinal endoscope, leaving the overtube. This enables the use of many commercially available accessories for ERCP. If rubber band is used to fix the endoscopic balloon, the rubber band will become stuck on the tip of the overtube when the DBE is removed, leaving the overtube. Thus, surgical thread should be used to fix the endoscopic balloon [17] (Fig. 2).

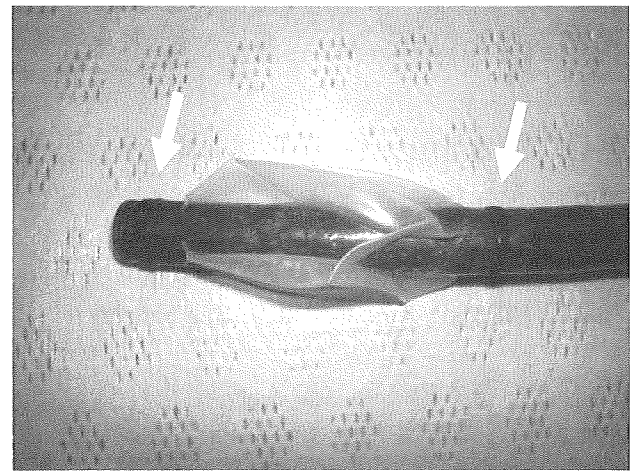

Fig. 2 A balloon is attached to the tip of the endoscope using surgical thread (*arrows*). From Ref. [17], with permission

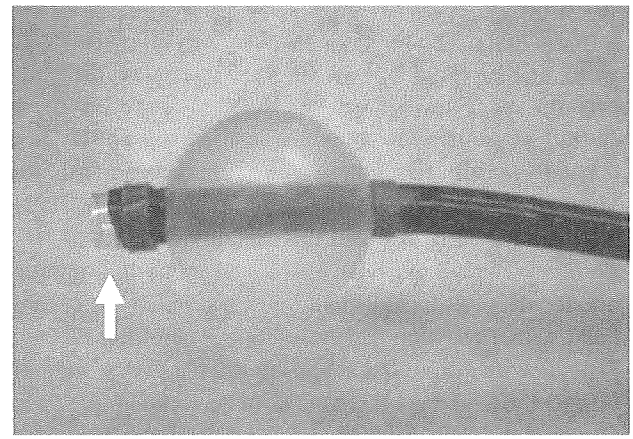

Fig. 3 A tip attachment (*arrow*) facilitates cannulation. From Ref. [17], with permission

If the "short" double-balloon endoscope EC-450BI5 is used, many commercially available accessories can be used for ERCP, because the diameter of the working channel is large, at 2.8 mm, and the working length is short, at 1520 mm. DBEs are not provided with an elevator, so cannulation may be difficult, but the preliminary use of a tip attachment (D-201-11304; Olympus Medical Systems, Tokyo, Japan; Fig. 3) before the DBE is inserted may facilitate cannulation.

Approach to the afferent loop

It is said that there is a large amount of bile in the direction of the afferent loop, and that the direction can be distinguished in accordance with the antiperistaltic motion of the intestinal tract, but in fact, the direction is often difficult to distinguish (Fig. 4a). If insertion of the DBE is mistakenly performed in the direction of the efferent loop, the injection of India ink into the small-intestinal mucosa becomes a marker so that the direction can be distinguished later (Fig. 4b). Thus, the characteristics of the DBE are made use of, and the DBE is inserted while aiming for the papilla of Vater or the bilio-enteric anastomosis as the intestinal tract is being shortened (Fig. 4c, d). In regard to the insertion of an endoscope and shortening of the intestinal tract, attention should be paid in order to avoid forced insertion or shortening. There is a risk of causing a perforation due to forced insertion or shortening [17].

Cannulation

In patients with Roux-en-Y reconstruction, the position of the papilla may vary and the position may be observed in various directions on a screen. In this procedure, first, the endoscope is axially rotated in order to position the papilla so that cannulation can be performed as easily as possible. The papilla can often be place only tangentially, but in such a case, the use of a tip attachment may facilitate cannulation. Next, regarding the catheter, we usually use a normal ERCP catheter. If the biliary axis does not fit by any means,

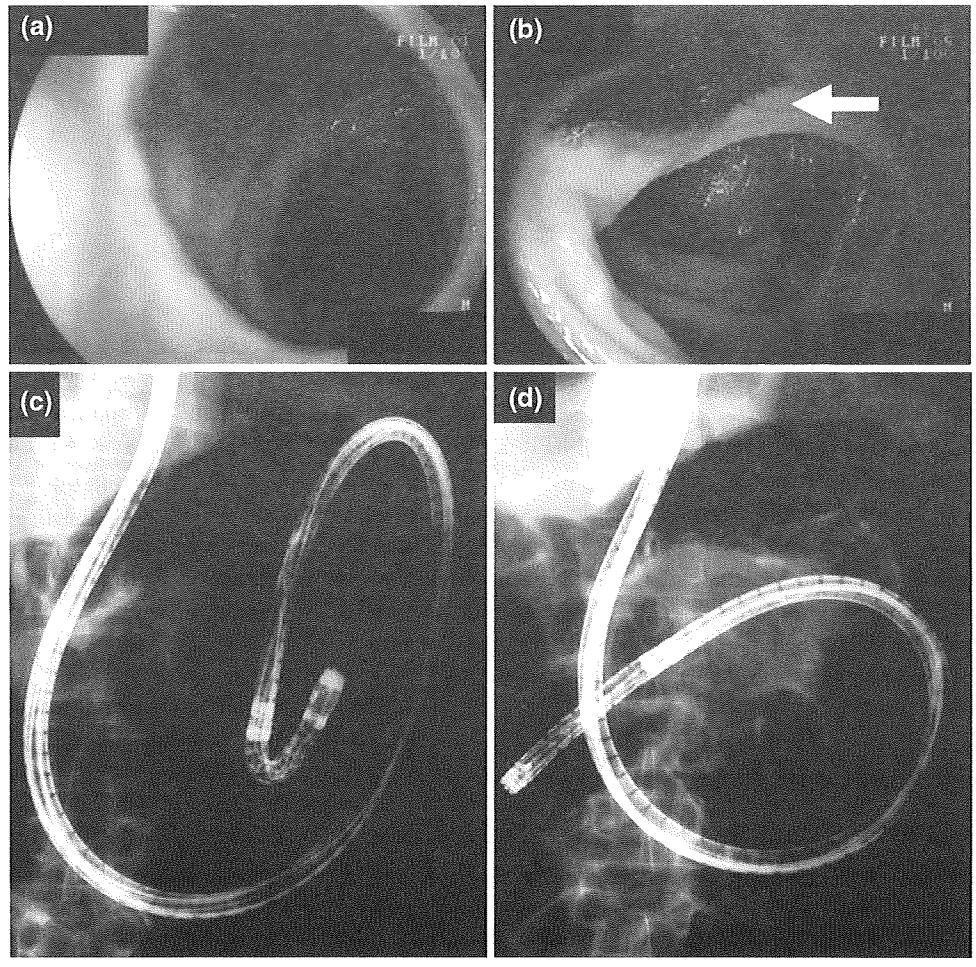

Fig. 4 **a** Junction of afferent loop and efferent loop. The direction of the afferent loop is often difficult to distinguish. **b** If we inserted the endoscope into the efferent loop by mistake, India ink was injected (*arrow*) so that the mistake would not be repeated. **c** The small bowel is elongated. **d** By using two balloons, one attached to the tip of the endoscope and the other at the distal end of an overtube, to grip the intestinal wall, the endoscope can be inserted further without forming redundant loops in the small intestine. From Ref. [17], with permission