recipients might be appropriate if the recipient outcomes are comparable to those for younger recipients and if donor morbidity is acceptable.

Thus, the aim of this study was to review the outcomes of LDLT for elderly recipients and to determine whether LDLT can be used with acceptable outcomes. We also reviewed the outcomes of very old recipients who were 70 years old or older.

PATIENTS AND METHODS

Patients

Between May 1997 and May 2013, adult-to-adult LDLT was performed 415 times at Kyushu University Hospital (Fukuoka, Japan). After the exclusion of auxiliary LDLT (n = 2), dual-graft LDLT (n = 1), and domino LDLT with a whole liver (n = 1), 411 LDLT cases using standard partial graft implantation techniques were included in the following analyses. All LDLT procedures were performed after informed consent was obtained from the patients and approval was obtained from the LT committee and institutional review board of Kyushu University. The mean follow-up time was 5.5 ± 3.5 years. Elderly recipients were defined as patients who were 65 years old or older according to the definition used by the US Census Bureau in 2010.[9] This study protocol was approval by institutional review board.

Indications for LDLT

LDLT is indicated for acute liver failure and chronic liver diseases with or without hepatic malignancies.[10] A recipient eligible for LDLT needs to satisfy the following criteria: (1) a high likelihood of having a healthy daily life after successful LDLT; (2) LDLT is the only treatment option to save the patient's life; (3) the patient's vital organs, other than the liver, show well-preserved function; (4) there is no uncontrollable malignancy or active infection in any organ except for the liver; (5) the patient is not dependent on drugs or alcohol; and (6) the patient and the patient's supporting family members are expected to show good compliance with medical management. Currently, the upper age limit for LDLT is 75 years. Recipients who are less than 70 years old must meet the same criteria for LDLT as younger recipients at our institute. Recipients who are 70 years old or older must meet additional criteria, including a 0-3/4 performance status and a Model for End-Stage Liver Disease (MELD) score ≤ 20; these are added to the aforementioned universal criteria for those with chronic liver disease. For recipients with acute liver failure who are 70 years old or older, a 0-1/4 Karnofsky performance status before the acute illness and the universal criteria are the criteria.[11] However, the actual indication is fully discussed on a case-by-case basis. A patient with dementia (not hepatic encephalopathy) is not eligible for LDLT because of unpreserved function of the brain (a vital organ) and poor posttransplant compliance.

The cardiovascular workup for an LDLT candidate includes an evaluation of the patient's medical history, a physical examination, electrocardiography, and 2-dimensional echocardiography. The cerebrovascular workup includes magnetic resonance imaging angiography and Doppler ultrasound. Pretransplant cancer screening for all recipient candidates includes whole-body computed tomography with an intravenous contrast medium, upper gastrointestinal endoscopy, and colonoscopy.

At Kyushu University Hospital, donors are limited to spouses or third-degree relatives by blood.[10] Donors need to be healthy without any medical problems, including diabetes, hypertension, bronchial asthma, and liver dysfunction. LDLT donors must be 20 to 65 years old.

We indicate LDLT for patients with hepatocellular carcinoma (HCC) beyond the Milan criteria. Only patients with a des-gamma-carboxyprothrombin level > 300 mAU/mL and a maximum tumor size > 5 cm are contraindicated, as previously reported.[12]

Grafts are selected as previously described.[13,14] Briefly, left lobe grafts are considered to be the primary graft type if the desired graft volume (GV)/standard liver volume (SLV) ratio is $\geq 35\%$. Right lobe grafts are considered if the simulated GV/SLV ratio for the left lobe graft is $<35\%$ and the donor's remnant liver volume will be $\geq 35\%$. The surgical procedures for donors and recipients have been described in our prior reports.[15]

After LDLT, patients are followed up by our outpatient department every week for 1 month, every month for 6 months, and every 3 months thereafter.

Immunosuppression Protocol

The basic immunosuppression protocol consists of tacrolimus or cyclosporine with mycophenolate mofetil and steroids. The target tacrolimus level is 10 to 15 ng/mL 1 month after LDLT and is decreased to 5 to 10 ng/mL over the next few months. The target cyclosporine level is 200 to 250 ng/mL 1 month after LDLT, and it is decreased to 100 to 200 ng/mL over the next few months. For elderly recipients, tacrolimus and cyclosporine levels more than half a year after LDLT are maintained at 3 to 7 and 70 to 120 ng/mL, respectively. One gram of methylprednisolone is given after reperfusion, and this is decreased from 200 to 20 mg daily over the course of 1 week; then, a switch is made to oral prednisolone, which is tapered off at 3 months.

Statistical Analysis

Values are expressed as means and standard deviations. Variables were analyzed with the χ^2 test (categorical values) or the Mann-Whitney test (continuous variables). Cumulative survival analyses were made with the Kaplan-Meier method with a log-rank test. P values < 0.05 were considered significant. All

TABLE 1. Donor and Recipient Characteristics

Factor	Younger Group: <65 Years Old (n = 365)	Elderly Group: ≥65 Years Old (n = 46)	*P* Value
Recipient age (years)*	49.8 ± 11.2	67.0 ± 2.2	<0.001
Recipient sex: male [n (%)]	177 (48.5)	15 (32.6)	0.04
Height (cm)*	161.1 ± 8.4	154.5 ± 7.3	<0.001
Body weight (kg)*	60.7 ± 11.1	55.6 ± 8.3	<0.01
Body mass index (kg/m^2)*	23.3 ± 2.5	23.2 ± 3.5	0.83
Body surface area (m^2)*	1.63 ± 0.19	1.53 ± 0.14	<0.001
SLV (mL)*	1156 ± 119	1081 ± 98	<0.001
Child class C [n/N (%)]	204/307 (66.4)	22/44 (50.0)	0.03
MELD score*	17.5 ± 7.2	14.8 ± 4.9	0.02
MELD score > 20 [n (%)]	114 (31.2)	7 (15.2)	0.01
Acute liver failure [n (%)]	58 (15.9)	2 (4.3)	0.04
Hepatitis C [n (%)]	135 (37.0)	33 (71.7)	<0.001
HCC [n (%)]	138 (37.8)	35 (76.1)	<0.001
Diabetes mellitus [n (%)]	63 (17.3)	10 (21.7)	0.45
Hospitalized [n (%)]	178 (48.8)	13 (28.3)	<0.01
Donor age (years)*	38.0 ± 4.7	36.3 ± 11.8	0.32
Donor sex: male [n (%)]	130 (35.6)	15 (32.6)	0.68
ABO-incompatible donor [n (%)]	18 (4.9)	2 (4.3)	0.86
Left lobe graft [n (%)]	218 (59.7)	37 (80.4)	0.02
GV (g)*	484 ± 108	433 ± 88	<0.001
GV/SLV (%)*	41.9 ± 8.7	40.3 ± 8.2	0.22
Graft-to-recipient weight ratio (%)*	0.81 ± 0.19	0.79 ± 0.18	0.50

*The data are presented as means and standard deviations.

statistical analyses were performed with JMP 7.0.1 (SAS Institute, Inc., Cary, NC).

RESULTS

Recipient and Donor Characteristics

The mean age of the recipients was 49.8 ± 11.2 years for the younger group (<65 years old, n = 365) and 67.0 ± 2.2 years for the elderly group (≥65 years, n = 46; $P < 0.001$; Table 1). There was a higher proportion of females in the elderly group (67.4% versus 51.5%, $P = 0.04$). The height (154.5 ± 7.3 cm for the elderly group versus 161.1 ± 8.4 cm for the younger group, $P < 0.001$), body weight (55.6 ± 8.3 versus 60.7 ± 11.1 kg, $P = 0.01$), body surface area (1.53 ± 0.14 versus 1.63 ± 0.19 m^2, $P < 0.001$), and SLV (1081 ± 98 versus 1156 ± 119 mL, $P < 0.001$) were lower for the elderly group. The proportion of Child class C patients [after the exclusion of patients with acute liver failure (n = 60)] was lower for the elderly group (50.0% versus 66.4%, $P = 0.03$). The MELD scores were also lower for the elderly group (14.8 ± 4.9 versus 17.5 ± 7.2, $P = 0.02$). Additionally, there were fewer hospitalized patients in the elderly group (28.3% versus 48.8%, $P < 0.01$). Histograms revealed that the elderly group included a lower proportion of patients with MELD scores > 20 (15.2% versus 31.2%, $P = 0.016$) and more Child class B patients (45.4% versus 24.8%, $P < 0.01$; Fig. 1). The elderly group was more likely to have hepatitis C (71.7% versus 36.9%, $P < 0.001$) or HCC (76.1% versus 37.8%, $P < 0.001$) and was less likely to have acute liver failure (4.4% versus 15.9%, $P = 0.036$). As for patients with HCC in the elderly and younger groups, 65.7% and 63.7%, respectively, were within the Milan criteria.

There were no significant differences in donor age or sex distribution between the elderly and younger groups. The donors for the elderly recipients were either sons (n = 31) or daughters (n = 15) of the recipients. Left lobe grafts (80.4% versus 59.7%, $P = 0.021$) with smaller GVs (433 ± 88 versus 484 ± 108 g, $P < 0.001$) were more frequent for the elderly group versus the younger group, but the GV/SLV ratios were similar for the 2 groups.

Operative and Postoperative Outcomes

There were no differences in operative outcomes except for shorter cold ischemia times in the elderly group (Table 2). In terms of postoperative outcomes, the frequency of acute cellular rejection (ACR) was lower for the elderly group (4.3% versus 16.2%, $P = 0.03$). However, the frequency of temporary neuropsychiatric complications was higher for the elderly group (32.6% versus 17.3%, $P = 0.01$), and these complications included delirium (n = 7), mutism (n = 3), loss of consciousness (n = 2), disorientation (n = 1), severe tremor (n = 1), and intractable headache (n = 1). There were no significant differences in other complications, including infections, between the 2 groups. The postoperative hospital stays were also similar for the younger and elderly groups (43.7 ± 33.1 versus 38.9 ± 26.8 days, $P = 0.35$).

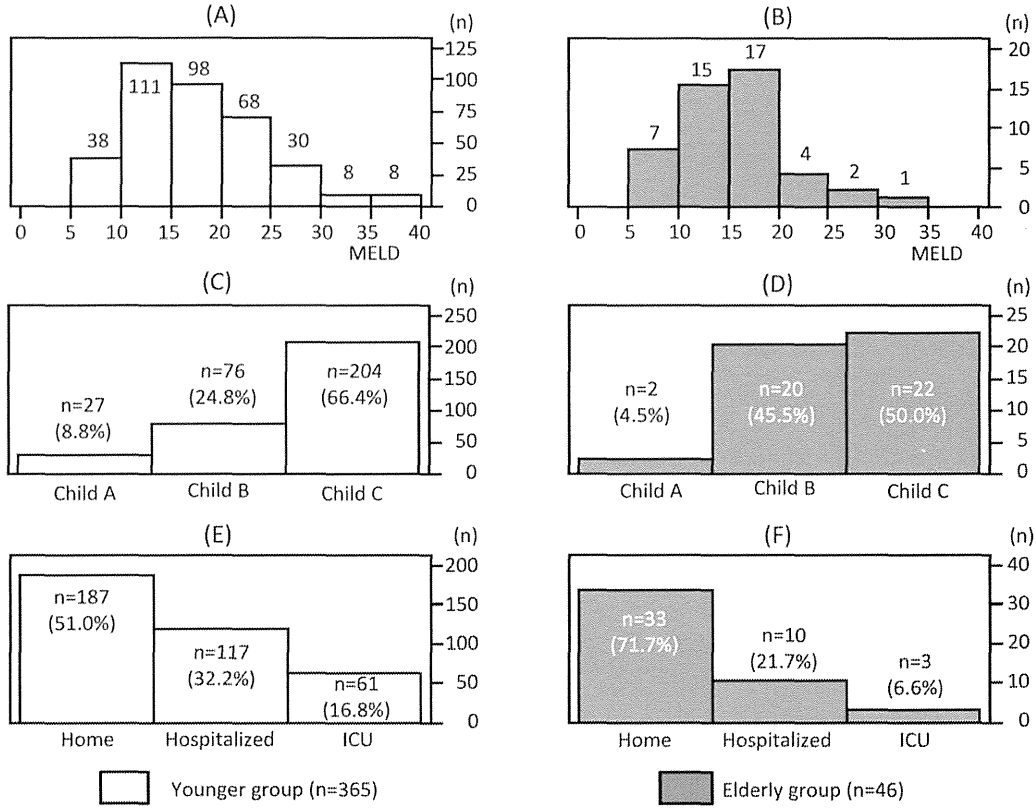

Figure 1. Distributions of (A,B) MELD scores, (C,D) Child classes, and (E,F) patient statuses before LDLT.

The 1-, 5-, and 10-year cumulative graft survival rates were 95.4%, 89.8%, and 77.8%, respectively, for the elderly group and 87.2%, 79.3%, and 72.9%, respectively, for the younger group ($P = 0.21$). The 1-, 5-, and 10-year patient survival rates were 93.2%, 82.8%, and 66.2%, respectively, for the elderly group

TABLE 2. Operative and Postoperative Outcomes

Factor	Younger Group: <65 Years Old (n = 365)	Elderly Group: ≥65 Years Old (n = 46)	*P* Value
Portal vein pressure at laparotomy (mm Hg)*	24.0 ± 6.1	23.7 ± 5.4	0.72
Portal vein pressure at closure (mm Hg)*	16.8 ± 4.4	16.8 ± 4.2	0.98
Cold ischemia time (minutes)*	96.1 ± 60.7	71.9 ± 32.8	<0.01
Warm ischemia time (minutes)*	41.3 ± 12.7	39.0 ± 13.1	0.24
Hepatic artery flow (mL/minute)*	121 ± 99	103 ± 45	0.27
Portal vein flow (L/minute)*	1.64 ± 0.66	1.59 ± 0.58	0.63
Splenectomy [n (%)]	179 (49.0)	23 (50.0)	0.90
Operation time (minutes)*	802 ± 182	760 ± 152	0.14
Blood loss (L)*	7.4 ± 15.2	4.6 ± 4.6	0.22
Primary graft dysfunction [n (%)]	47 (12.9)	2 (4.3)	0.10
Total bilirubin on day 14 (mg/dL)*	7.6 ± 8.5	5.0 ± 5.3	0.04
Ascites output on day 14 (L/day)*	0.55 ± 0.94	0.29 ± 0.48	0.08
ACR [n (%)]	59 (16.2)	2 (4.3)	0.03
Hepatic artery thrombosis [n (%)]	7 (1.9)	0 (0.0)	0.34
Portal venous thrombosis [n (%)]	8 (2.2)	0 (0.0)	0.31
Cytomegalovirus infection [n (%)]	89 (24.4)	10 (21.7)	0.69
Bacterial sepsis [n (%)]	49 (13.4)	2 (4.3)	0.08
Neuropsychiatric complications [n (%)]	63 (17.3)	15 (32.6)	0.01
Biliary stenosis [n (%)]	69 (18.9)	13 (28.3)	0.14
Hospital stay (days)*	43.7 ± 33.1	38.9 ± 26.8	0.35

*The data are presented as means and standard deviations.

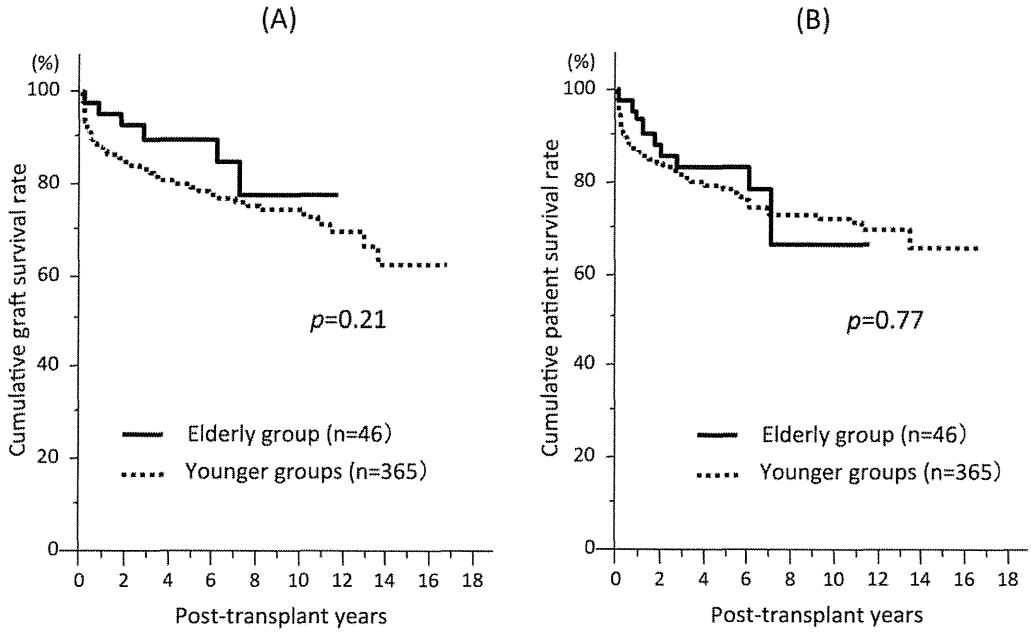

Figure 2. (A) Cumulative graft survival and (B) cumulative patient survival after LDLT for the elderly group (n = 46) and the younger group (n = 365).

and 86.8%, 78.0%, and 72.0%, respectively, for the younger group ($P = 0.77$; Fig. 2).

Causes of Death

Overall, 88 patients (24.1%) in the younger group and 9 patients (19.6%) in the older group died during the observation period. In the younger group, the main causes of death were recurrent malignancies (18.2%), sepsis (17.0%), and primary graft dysfunction (12.4%). The main causes of death for the elderly group were de novo malignancies, including lung cancer (n = 1) and leukemia (n = 1); recurrent HCC (n = 2); and other causes, including disappearance due to dementia (n = 1), drowning in a bathtub (n = 1), and chronic pulmonary obstructive disease (n = 1). Cerebrovascular events included subarachnoid hemorrhage (n = 1) and cerebral hemorrhage (n = 1) and only occurred in the younger group. No cardiovascular events occurred in either group. The causes of death were not significantly different between the 2 groups.

Outcomes for Recipients 70 Years Old or Older

Seven of the recipients were 70 years old or older (Table 3). They were indicated for LDLT for hepatitis C with or without HCC. The mean MELD score was 15.6 ± 5.2, and 6 of the patients were treated as outpatients before LDLT. Because all the donors were children of the recipients, the average donor age was approximately 43 years. The mean GV/SLV ratio was 44.1% ± 2.2%. Only 1 recipient had a high MELD score of 26, and she was treated with plasmapheresis in an intensive care unit for acute deterioration of chronic hepatitis C after open cholecystectomy and choledochotomy. However, this patient was well oriented and was self-supported at home before the insult prompting the indication for LDLT. Although 3 of the 7 patients experienced delirium after LDLT, all survived and showed good compliance with medical management.

DISCUSSION

This study was conducted to determine whether LDLT was appropriate for elderly recipients in terms of the outcomes of both recipients and donors. We found that elderly but low-risk patients were indicated for LDLT, which resulted in fairly acceptable mid- and long-term outcomes. However, the selection bias did not confer better graft survival to the elderly recipients. Very old recipients who were 70 years or older under select conditions also showed acceptable survival after LDLT.

There was inevitably some selection bias in this study because LDLT is usually performed after the determination of whether the risk-benefit balance is appropriate.[16,17] A randomized study evaluating the feasibility of LDLT in older recipients is ethically unfeasible. Therefore, we believe that the elderly patients included in this series were appropriately selected, and acceptable patient outcomes resulted. There may also be some bias for performing LDLT for sicker young recipients even if the outcomes might be less favorable. Donors also face surgical risk, which is important because of the emotional link between donors and recipients.

Several studies have focused on DDLT in elderly recipients, with opinions changing over time. The reports published in the early 1990s revealed no decrease in survival among older LT recipients.[18,19] However, since then, researchers have raised caution about indications for DDLT in high-risk elderly

TABLE 3. Characteristics of Recipients 70 Years Old or Older

Recipient Age (Years)/Sex	Disease	MELD Score	Child Class	Status	Donor Age (Years)/Sex	Relationship	Graft	GV/SLV Ratio (%)	Complications	Outcomes
70/female	Hepatitis C, HCC	18	B	Home	44/male	Son	Left	45.9	Delirium	Alive, 11.5 years
70/female	Hepatitis C, HCC	21	C	Home	39/male	Son	Right	47.3	No	Alive, 9.6 years
73/female	Hepatitis C, HCC	14	B	Home	46/male	Son	Left	40.4	Biliary anastomosis stricture	Alive, 6.3 years
71/female	Hepatitis C, HCC	9	B	Home	40/female	Daughter	Left	34.1	No	Alive, 5.3 years
72/female	Hepatitis C, HCC	9	B	Home	43/male	Son	Left	40.9	Delirium	Alive, 3.3 years
70/female	Hepatitis C	12	B	Home	43/female	Daughter	Right	48.8	Delirium, biliary anastomosis stricture	Alive, 2.8 years
70/female	Hepatitis C (acute-on-chronic)	26	C	Intensive care unit	47/female	Daughter	Right	51.1	No	Alive, 1.0 year

recipients because early postoperative outcomes were dismal.[6,20] Zetterman et al.[20] evaluated 735 cases of DDLT, which included younger recipients (<60 years old) and older recipients (≥60 years old) with similar disease severity, and they showed that older recipients had significantly worse short-term graft survival. Thereafter, Levy et al.[5] reported that hospitalized elderly patients had significantly worse short-term graft outcomes, whereas low-risk elderly patients had outcomes similar to those of younger recipients. Garcia et al.[21] also reported that the risk of graft loss for Child class C patients was significantly greater for patients older than 65 years. In subsequent reports, low-risk elderly recipients with low MELD scores and a Child class less than C were selected for LT and compared with younger patients. Cross et al.[22] reported that the 5-year patient survival rates were comparable for elderly and young recipients despite the presence of a selection bias. Thus, many transplant centers have became aware that LT should not be denied to elderly patients on the basis of their age alone as long as the patients are appropriately selected in terms of less advanced liver disease and the absence of comorbidities.

It is well accepted that the graft quality, including the graft size and the donor age, and the recipient condition, including the patient status, the MELD score, and the extent of portal hypertension, are determinants of recipient outcomes.[23] We previously reported that advanced donor age, recipient hospitalization, and high MELD scores were among the risk factors for postoperative primary graft dysfunction with severe hyperbilirubinemia.[24] In the current study, all of the donors for the elderly recipients were their children. Therefore, the donors were relatively young, in their 30s or 40s, and had good hepatic function. Moreover, the elderly recipients had a smaller body size with a smaller SLV, and this resulted in a greater likelihood of an acceptable GV/SLV ratio despite their smaller GV.[25] However, it seems that the recipient condition is the main determinant of the indication for LDLT and its outcomes in elderly recipients, just as for DDLT. Even without accounting for recipient age, we found hospitalization and a high MELD score to be significant risk factors for graft dysfunction and mortality.[24] We would probably not consider LDLT for elderly hospitalized patients with a 4/4 performance status and a MELD score > 20.

The most frequent cause of death for the elderly recipients was de novo or recurrent malignancy, although the incidence of these events was low. This increased risk of malignancy-related death is unsurprising because the risk of malignancies increases with age.[26-28] Herrero[27] reviewed more than 300 cases of LT and showed that de novo malignancy was the leading cause of death 3 years after LT, and the risks for malignancy were advanced age, smoking, Epstein-Barr virus, and sun exposure. Preventive measures included sunlight protection, smoking cessation, and the avoidance of overimmunosuppression. Because

aging itself cannot be changed, general cancer screening seems to be the only way of detecting malignancies at an early stage of disease.

Another interesting observation from this study was the lower incidence of ACR in the elderly recipients (4.3%) versus the younger recipients (16.2%). Similar results were reported for DDLT: the incidence of ACR was 43% for recipients who were 65 years old or older and 61% for recipients who were less than 60 years old. Although it is debated whether the ACR rate differs between LDLT and DDLT,[29] Liu et al.[30] reported that the incidence of ACR was significantly lower with LDLT using related donors versus LDLT using unrelated donors. A result of aging is a reduction of the cellular and humoral immune responses.[31] Immune senescence could be a double-edged sword: it is associated with a decreased risk of rejection and an increased risk of infection and malignancy. However, because the development of a severe infection is often associated with early graft function and the recipient status before transplantation, the aged immune system could decrease the likelihood of early ACR but increase the likelihood of late de novo malignancy.

The main limitation of this study was the strong selection bias. The elderly recipients had lower MELD scores, more were Child class B, and they had a better performance status without hospitalization. This patient population is characterized by a high risk of HCC after liver cirrhosis caused by hepatitis C, which was the main indication for the elderly group. Although a large multicenter study might be able to overcome these limitations, a similar selection bias may exist in other studies because of the nature of LDLT, which imposes a surgical risk on the donors. Despite this limitation, single-center studies allow researchers to evaluate subjects in more detail and maintain consistent patient management and surgical procedures.

The other significant limitation of this study is the use of 65 years as the definition for elderly recipients in accordance with the US Census Bureau in 2010.[9] Because of the increase in the average lifespan worldwide, the clinical outcomes of septuagenarian LDLT recipients could be more informative. However, the selection bias for such recipients, due to the risks for the donors, limited the number of septuagenarian recipients, so there was not enough power for statistical analyses. Future accumulations of such recipients or multicenter studies could help.

In conclusion, LDLT is appropriate for low-risk elderly recipients and has favorable long-term outcomes for these recipients.

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ORIGINAL ARTICLE

A high MELD score, combined with the presence of hepatitis C, is associated with a poor prognosis in living donor liver transplantation

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Abstract
Purpose The feasibility of performing living donor liver transplantation (LDLT) for patients with high end-stage liver disease (MELD) scores needs to be assessed.
Methods A total of 357 patients who underwent LDLT were included in this analysis.
Results Overall, 46 patients had high MELD scores (≥25) and their graft survival was similar to that in patients with low MELD scores (<25; $n = 311$; $p = 0.395$). However, among patients with high MELD scores, a multivariate analysis showed that the presence of hepatitis C ($p = 0.013$) and LDLT in Era-I ($p = 0.036$) was significantly associated with a poorer prognosis. Among patients with hepatitis C ($n = 155$), the 5-year graft survival rate was significantly lower in patients with high MELD scores (33.7 %, $p < 0.001$) than in patients with low MELD scores. The 5-year graft survival rate was significantly lower in patients in Era-I ($n = 119$) compared with those in Era-II/III when stratified by low (73.0 vs. 82.5 %, $p = 0.040$) and high (55.0 vs. 86.1 %, $p = 0.023$) MELD scores. Among the patients with high MELD scores, those with hepatitis C and LDLT in Era-I had the worst 5-year graft survival rate (14.3, $p < 0.001$).
Conclusion The graft outcomes in patients with high MELD scores and the presence of hepatitis C were found to be particularly poor.

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Keywords Living donor liver transplantation · Hepatitis C · Model for end-stage liver disease · Learning curve

Abbreviations

GRWR	Graft recipient weight ratio
GV	Graft volume
GW	Graft weight
LDLT	Living donor liver transplantation
MELD	Model for end-stage liver disease
PVF	Portal venous flow
PVP	Portal venous pressure
SLV	Standard liver volume

Introduction

The model for end-stage liver disease (MELD) was originally developed as a scoring system to assess the severity of terminal liver diseases. Therefore, it is often used as part of the criteria for allocating deceased donor livers [1, 2]. Previous studies have shown that the MELD system might also predict graft outcomes after deceased donor liver transplantation (DDLT), although this possibility is still widely debated [3–5].

Partial grafts are always used in living donor liver transplantation (LDLT), but might be too small to fulfill the recipient's metabolic needs [6]. Therefore, the pre-transplant disease severity, as represented by a high MELD score, might be an important determinant of the graft outcome [7]. The technical advances in LDLT in the last decade have dramatically improved the overall graft outcomes after LDLT [8–10]. The Toronto group recently reported that LDLT could provide excellent graft outcomes, even in patients with high MELD scores [11].

However, the impact of high MELD scores on the outcome of LDLT has not been fully elucidated, and is hotly debated [7–11]. Moreover, there has so far been no subgroup analyses of patients with high MELD scores aimed at elucidating the factors associated with the graft outcomes after LDLT.

Therefore, the aims of this study were to evaluate the overall impact of the MELD score on the graft outcomes in LDLT, and to identify clinically relevant prognostic factors in patients with high MELD scores.

Materials and methods

Patients

We retrospectively analyzed our prospective database of all adult-to-adult LDLTs performed since May 1997 ($n = 357$). The recipients included 172 males (48.2 %), and the mean age of the recipients was 51.6 ± 11.6 years. Hepatitis C infection was present in 155 (43.4 %) patients, and hepatocellular carcinoma was present in 156 (43.8 %). The primary liver diseases included liver cirrhosis ($n = 216$; hepatitis C, $n = 153$; hepatitis B, $n = 40$), cholestatic liver diseases ($n = 78$), acute liver or graft failure ($n = 54$; including hepatitis B, $n = 17$; hepatitis C, $n = 2$; hepatic artery thrombosis, $n = 1$; graft congestion, $n = 1$; primary graft failure, $n = 1$) and others ($n = 8$). A major shunt vessel was defined as a portosystemic shunt with a caliber >10 mm.

The donors included 231 males (64.8 %), and the mean age of the donors was 35.9 ± 11.1 years. Seventeen (4.8 %) donors were blood-type incompatible donors. The graft types included left lobe ($n = 223$, 62.6 %), right lobe ($n = 128$, 35.8 %) and posterior segment ($n = 6$, 1.7 %) grafts. The mean graft volume (GV), graft volume/standard liver volume (GV/SLV) ratio and graft recipient weight ratio (GRWR) were 479 ± 106 g, 41.7 ± 8.5 % and 0.81 ± 0.19. All of the LDLTs were performed after obtaining full informed consents from all patients and approval from the Liver Transplantation Committee of Kyushu University. The mean follow-up time was 4.8 ± 3.2 years.

MELD score

The pretransplant total bilirubin levels, creatinine levels and prothrombin time international normalized ratio (PT-INR) were used to calculate the medical MELD score without the additional MELD points [1]. A high MELD score is not a contraindication for LDLT at our center.

Graft selection and surgical procedures

The grafts were selected as described previously [12]. Left lobe grafts were considered to be the primary graft type if the desired GV/SLV was >35 %. Right lobe grafts were considered if the simulated GV/SLV of the left lobe graft was <35 % and the donor's remnant liver volume was >35 %.

The surgical procedures used are described elsewhere [12]. Briefly, the procured graft was perfused ex situ using University of Wisconsin solution (Viaspan™, DuPont Inc., Wilmington, DE). Splenectomy was performed to control the portal venous pressure after reperfusion or to treat thrombocytopenia before introducing interferon treatment for recurrent hepatitis C, if indicated [13].

Immunosuppression and anti-viral treatment for hepatitis C

The immunosuppression protocol consisted of tacrolimus or cyclosporine with mycophenolate mofetil and steroids [12]. The antiviral treatment for hepatitis C consisted of pegylated interferon α2a or 2b plus ribavirin [14].

Assessment of outcomes after LDLT

The endpoint of this study was graft loss, including patient death or re-transplantation. Deaths caused by infection, cardiovascular diseases or recurrent hepatocellular carcinoma were included as graft loss. However, deaths caused by de novo malignancies or accidents were censored.

Transplant era

The total cohort of 357 patients was divided into three groups of equal numbers of consecutively treated patients, Era-I ($n = 119$) consisted of patients 1–119 who were treated between May 1997 and February 2004, Era-II ($n = 119$) consisted of patients 120–238 who were treated between March 2004 and January 2008 and Era-III ($n = 119$) consisted of patients 239–357 who were treated since February 2009.

Statistical analysis

The values are expressed as the mean ± standard deviation or as n (%). Variables were analyzed using the χ^2 tests for categorical variables and the Mann–Whitney U test for continuous variables. The univariate and multivariate survival analyses were performed using the Kaplan–Meier method with the log-rank test and Cox proportional hazards model, respectively. Values of $p < 0.05$ were considered to be statistically significant.

Results

Surgical and postoperative outcomes

The 1- and 5-year cumulative graft survival rates were 87.1 and 78.2 %, respectively. The recipient and donor graft variables, and post-transplant characteristics, are summarized in Table 1.

MELD score and graft survival

A number of patients with MELD scores of <5, 5–9, 10–14, 15–19, 20–24 and ≥25 were 0 (0.0 %), 41 (11.5 %), 108 (30.3 %), 94 (26.3 %), 68 (19.1 %) and 46 (12.8 %), respectively (Fig. 1a). The median and the mean MELD scores were 16 and 17.1 ± 6.9, respectively. The 5-year graft survival rates in the patients with MELD scores of <5 ($n = 148$), 5–25 ($n = 163$) and ≥25 ($n = 46$) were 79.9, 78.2 and 72.1 %, respectively ($p = 0.395$, Fig. 1b).

Characteristics of patients with high MELD scores

The patients were categorized into those with high (≥25, $n = 46$) or low (<25, $n = 311$) MELD scores. Patients with high MELD scores had significantly higher total bilirubin levels (20.8 ± 11.40 vs. 6.0 ± 7.0 mg/dl, $p < 0.001$), prolonged PT-INR (2.54 ± 1/17 vs. 1.48 ± 0.27, $p < 0.001$) and higher creatinine levels (0.8 ± 0.5 vs. 1.3 ± 1.4, $p < 0.001$). After LDLT, the incidence of cytomegalovirus infection (43.4 vs. 23.0 %, $p = 0.003$), bacterial sepsis (28.2 vs. 12.1 %, $p = 0.003$) and the peak total bilirubin levels

Table 1 Patient characteristics stratified by MELD score

Variables	MELD score		*p* value
	Low (<25, $n = 311$)	High (≥25, $n = 46$)	
MELD score	15.2 ± 4.6	30.1 ± 5.6	<0.001
Total bilirubin before LDLT	6.0 ± 7.0	20.8 ± 11.40	<0.001
PT-INR before LDLT	1.48 ± 0.27	2.54 ± 1.17	<0.001
Creatinine before LDLT (mg/dl)	0.8 ± 0.5	1.3 ± 1.4	<0.001
Donor age (years)	35.9 ± 11.4	35.6 ± 9.5	0.809
Donor gender, male	203 (65.5)	28 (60.9)	0.540
Incompatible blood type	17 (5.5)	0 (0.0)	0.104
Left lobe graft	198 (63.8)	25 (54.3)	0.795
GV (g)	478 ± 102	489 ± 127	0.481
GV/SLV ratio (%)	41.6 ± 8.4	42.3 ± 9.6	0.598
GRWR (%)	0.81 ± 0.19	0.83 ± 0.19	0.382
Recipient age (years)	52.2 ± 11.5	47.9 ± 12.2	0.230
Recipient gender, male	149 (48.1)	23 (50.0)	0.806
Hepatocellular carcinoma	153 (49.3)	3 (6.5)	<0.001
Hepatitis C	142 (45.5)	13 (28.3)	0.028
Cold ischemic time (min)	86.9 ± 54.9	95.2 ± 57.9	0.351
Warm ischemic time (min)	39.9 ± 11.9	39.0 ± 8.1	0.594
Hepatic arterial flow (ml/min)	106 ± 68	119 ± 56	0.231
Portal venous flow (l/min)	1.62 ± 0.65	1.54 ± 0.62	0.403
PVP at the closure (mmHg)	16.8 ± 4.4	17.2 ± 4.9	0.636
Major shunt vessels	62 (13.8)	6 (13.1)	0.266
Length of operation (min)	797 ± 174	796 ± 217	0.946
Intraoperative blood loss (l)	7.1 ± 15.4	7.2 ± 8.1	0.960
Acute cellular rejection	46 (14.9)	10 (21.7)	0.238
Hepatic artery thrombosis	6 (1.9)	1 (2.2)	0.918
Portal venous thrombosis	8 (2.6)	1 (2.2)	0.864
Cytomegalovirus infection	70 (23.0)	20 (43.4)	0.003
Pneumonia	36 (11.9)	10 (21.7)	0.067
Bacterial sepsis	37 (12.1)	13 (28.2)	0.003
Peak total bilirubin (mg/dl)	11.6 ± 9.7	17.3 ± 8.7	<0.001
Peak ascites output (l/day)	1.2 ± 1.4	1.3 ± 1.1	0.63

GRWR graft recipient weight ratio, *GV* graft volume, *LDLT* living donor liver transplantation, *MELD* model for end-stage liver disease, *POD* postoperative day, *PT-INR* prothrombin time international normalized ratio, *PVP* portal venous pressure, *SLV* standard liver volume

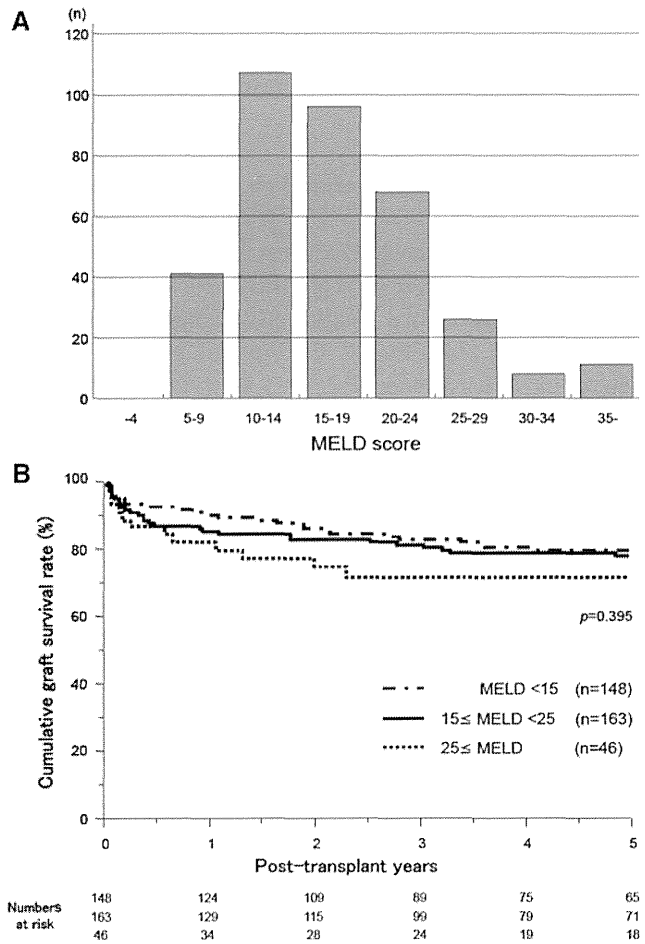

Fig. 1 Distribution of MELD scores (**a**), and the cumulative graft survival according to the MELD score (**b**)

(17.3 ± 8.7 vs. 11.6 ± 9.7, $p < 0.001$) were significantly higher in patients with high MELD scores.

We next evaluated the factors associated with graft loss among the patients with high MELD scores (≥25, $n = 46$). The univariate analysis showed that Era-I ($n = 119$, $p = 0.023$), recipient gender (male, $p = 0.045$), hepatitis C (positive, $p < 0.001$) and the presence of major shunt vessels (yes, $p = 0.010$) were significantly associated with early graft loss (Table 2). The multivariate analysis of these four factors showed that hepatitis C infection (yes, odds ratio 4.9, 95 % confidence interval 1.5–17.8, $p = 0.013$) and LDLT during Era-I (yes, odds ratio 4.0, 95 % confidence interval 1.2–15.8, $p = 0.036$) were independently associated with graft loss (Table 3).

Hepatitis C positive patients

The patients with hepatitis C were classified into four groups based on the MELD scores: <15 ($n = 82$), 15–19 ($n = 39$), 20–24 ($n = 21$) and ≥25 ($n = 13$). The 5-year graft survival rates in these four groups were 78.9, 80.0, 75.6 and 33.7 %, respectively. Patients with hepatitis C and MELD scores ≥25 had significantly worse graft outcomes compared with the other three groups ($p < 0.001$, Fig. 2a).

Among the patients without hepatitis C infection ($n = 202$), the 5-year survival rates in patients with low

Table 2 Results of the univariate analysis of graft mortality in patients with high (≥25) MELD scores

Variables	*n*	Graft survival rate (%)		
		1-year	5-year	*p* value
Era-I (first 1/3 cases)				
Yes	21	70.0	55.0	0.023
No	25	91.8	86.1	
Recipient gender, male				
Yes	23	77.3	54.1	0.045
No	23	86.5	86.5	
Emergency LDLT				
Yes	26	83.8	83.8	0.147
No	20	80.0	58.4	
Hepatitis C				
Yes	13	61.5	33.7	<0.001
No	33	90.4	86.6	
Donor age ≥40 years				
Yes	16	81.2	54.5	0.096
No	30	82.4	82.4	
Donor gender, male				
Yes	28	80.9	80.9	0.217
No	18	83.3	59.2	
Left lobe graft				
Yes	25	78.6	78.6	0.427
No	21	85.7	62.9	
GV/SLV <40 %				
Yes	21	88.0	84.1	0.623
No	25	91.7	72.4	
GRWR <0.8				
Yes	19	68.4	68.4	0.424
No	27	92.1	74.5	
Major shunt vessels				
Yes	6	50.0	33.3	0.010
No	40	84.1	77.9	
Splenectomy				
Yes	11	81.8	68.2	0.930
No	35	82.0	71.9	
Duct-to-duct				
Yes	16	75.0	66.8	0.686
No	43	90.1	80.8	

GRWR graft recipient weight ratio, *GV* graft volume, *LTLT* living donor liver transplantation, *MELD* model for end-stage liver disease, *SLV* standard liver volume

Table 3 Results of the multivariate analysis of graft mortality in patients with high (≥25) MELD scores

95 % confidence interval				
Variables	Odds ratio	Lower	Upper	*p* value
Hepatitis C	4.9	1.5	17.8	0.013
Era-I (first 1/3 cases)	4.0	1.2	15.8	0.036
Major shunt vessels	3.3	0.9	11.9	0.061
Recipient gender, male	3.1	0.8	12.2	0.106

MELD model for end-stage liver disease

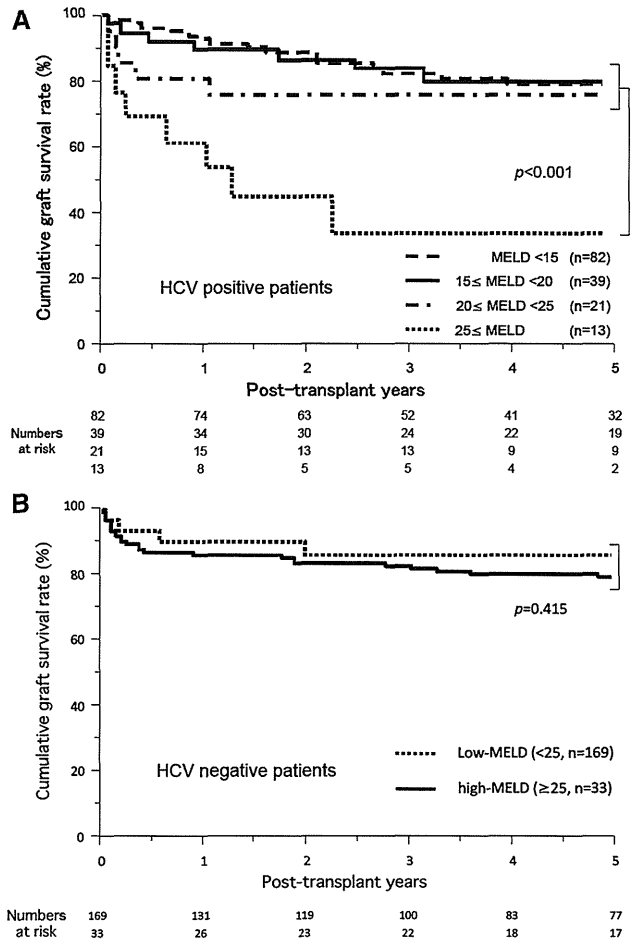

Fig. 2 Cumulative graft survival in patients with (**a**) or without (**b**) hepatitis C according to the MELD score

(<25, $n = 169$) and high (≥25, $n = 33$) MELD scores were 86.6 and 79.6 %, respectively ($p = 0.415$, Fig. 2b). Even when we excluded hepatitis C-negative patients with acute liver or graft failure from the analysis, the 5-year graft survival rates were comparable between those with low (<25, $n = 143$) and high (≥25, $n = 10$) MELD scores (81.5 and 80.0 %, respectively, $p = 0.926$). Therefore, hepatitis C was only associated with poor graft survival among the patients with high MELD scores.

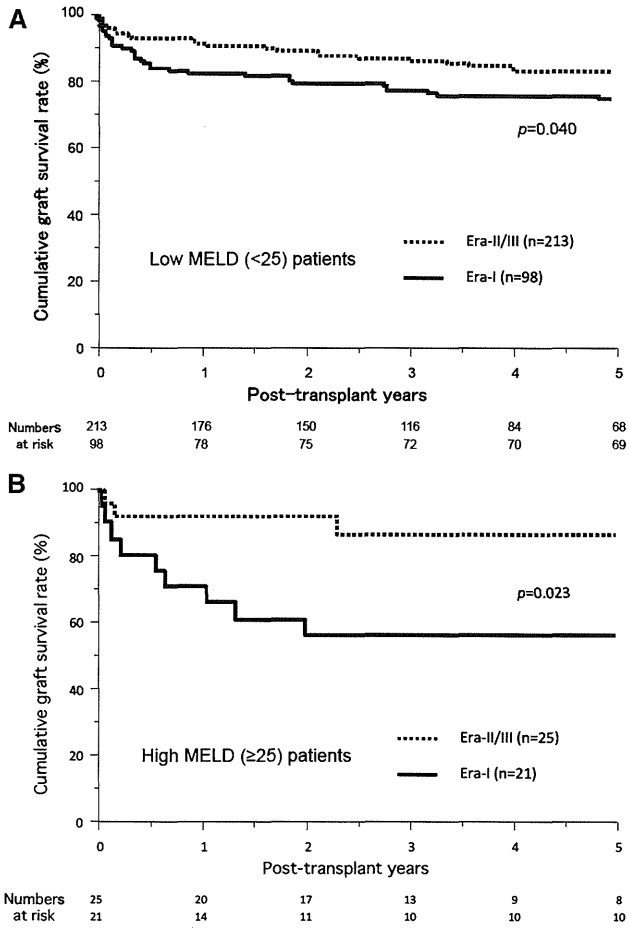

Fig. 3 Cumulative 5-year graft survival rate following LDLTs in Era-I (May 1997–February 2004) or Era-II/III (March 2004 onwards) in patients with low (**a**) or high (**b**) MELD scores

Transplant era and graft survival

The cumulative 5-year graft survival rate was compared between patients undergoing LDLT in Era-I or Era-II/III, and was stratified by high ($n = 46$) or low ($n = 311$) MELD scores. Among the patients with low MELD scores (Fig. 3a), the 5-year graft survival rate was significantly lower in patients who underwent LDLT in Era-I ($n = 98$), compared with Era-II/II ($n = 213$), with rates of 73.0 and 82.5 %, respectively ($p = 0.040$). The 5-year graft survival rate in patients with high MELD scores (Fig. 3b) was also significantly lower in the patients who underwent LDLT in Era-I ($n = 21$) than in Era-II/II ($n = 25$), with rates of 55.0 and 86.1 %, respectively ($p = 0.023$).

Effects of hepatitis C in combination with the transplant era

Patients with high MELD scores (≥25) were categorized into three groups according to the combination of time of

LDLT and hepatitis C status as follows: (1) LDLT in Era-II/III and absence of hepatitis C; (2) either LDLR in Era-I or the presence of hepatitis C; and (3) LDLT in Era-I and the presence of hepatitis C. The 5-year graft survival rates of these three groups of patients were 94.4, 72.6 and 14.3 %, respectively. Patients in group 3 (LDLT in Era-I and the presence of hepatitis C) had a significantly worse prognosis than those in the other two groups ($p < 0.001$). Among the patients with hepatitis C and high MELD scores who underwent LDLT in Era-I ($n = 7$), the causes of graft loss included graft dysfunction because of sepsis and multiple organ failure ($n = 3$), recurrent hepatitis C ($n = 2$) and recurrent hepatocellular carcinoma ($n = 1$). On the other hand, among patients with hepatitis C and high MELD scores who underwent LDLT in Era-II/III ($n = 6$), only one graft was lost because of recurrent hepatitis C. Although three out of the six (50 %) grafts in this group had aggressive recurrent hepatitis C, two patients underwent interferon treatment resulting in a viral response. Moreover, no grafts in patients with high MELD scores were lost as a result of septic complications in patients who underwent LDLT in Era-II/III (Fig. 4).

Discussion

The findings of the current study can be summarized as follows: first, the overall graft survival was not significantly different between patients with high or low MELD scores. Second, among patients with high MELD scores (≥25), the presence of hepatitis C and LDLT in Era-I (May 1997–February 2004) were significantly associated with a poor prognosis.

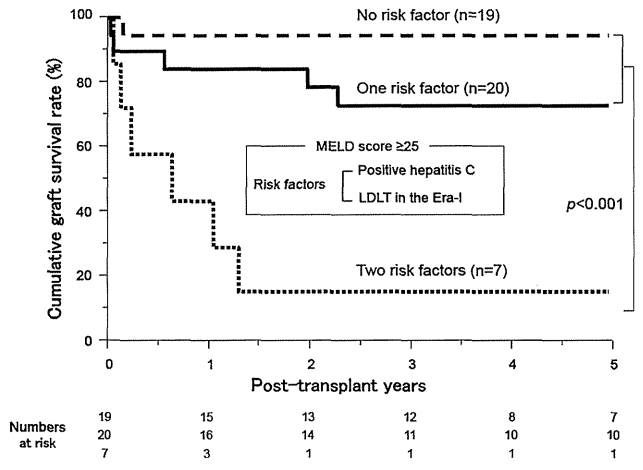

Fig. 4 Cumulative graft survival in three groups of patients with high MELD scores (≥25) stratified according to the time of LDLT and hepatitis C status: (1) LDLT in Era-II/II and the absence of hepatitis C ($n = 19$); (2) either LDLT in Era-I or the presence of hepatitis C ($n = 20$); and (3) LDLT in Era-I and the presence of hepatitis C ($n = 7$)

Regarding the overall general impact of high MELD scores, the current results appear to be convincing because it is generally accepted that surgical outcomes are largely influenced by the pre-surgical conditions [15]. However, the findings are reasonable considering the patient characteristics and transplant era, since the majority of patients had moderate MELD scores (median: 16, mean: 17) and most transplants were performed after 2000. On the other hand, the Kyoto group [10] analyzed 576 adult-to-adult cases since 1993, with a mean MELD score of 20, and found that patients with high MELD scores had an increased risk of graft loss (odds ratio 1.65). Their results are also reasonable, because their patients generally had higher MELD scores, and transplantation was done before 2000, before the introduction of major refinements in surgical techniques for adult-to-adult LDLT [16]. Marubashi et al. [7] reported similar results in their initial 39 cases with a higher mean MELD score of 22. In contrast, the Toronto group [11] recently reported a negative impact of the MELD score on graft outcomes. They analyzed more recent LDLTs since 2002 ($n = 271$); the mean MELD score of their patients was 17. Therefore, we would anticipate that our outcomes would be similar to those reported by the Toronto group. By taking into account these findings, it could be concluded that a high MELD score does not negatively affect the overall graft outcomes of patients undergoing LDLT in recent years, and with the application of the recent refinements in LDLT.

The negative effect of a high MELD score on graft outcomes in patients with hepatitis C patients is a particularly important finding. The difference in survival between patients with higher and lower MELD scores among those with hepatitis C became prominent within 3 months of LDLT, and the gap gradually increased with time, reaching 40 % 2 years after LDLT. The high risk of graft loss associated with a high MELD score and hepatitis C continues until 2 years after transplantation. This conflicts with the belief that the pre-transplant disease severity only affects graft outcomes in the very early post-transplant course, namely in the first 2–3 months after LDLT [10, 17]. In our patients, five out of 13 (38.5 %) with high MELD scores had aggressive recurrent hepatitis C, defined as cholestatic or fibrosing hepatitis C [14]. The incidence of aggressive hepatitis C was higher in patients with high MELD scores than in patients with low MELD scores (5/13 vs. 16/142, $p = 0.006$). Because there were no significant differences in the donor age, graft volume, immunosuppression protocol or viral load between patients with high or low MELD scores, the difference in the rate of aggressive hepatitis C might be attributed to the disease. However, there have so far been no reports describing an

association between the disease severity and progression of aggressive fibrosis. Retortillo et al. [18] reported that partial live donor grafts showed earlier fibrotic progression compared with deceased whole-liver transplants. Furthermore, Honda et al. [19] reported that hepatitis C virus actively infects and replicates in rapidly dividing hepatocytes via the activation of hepatocyte growth factors. A possible explanation for this could be that the metabolic demands on partial grafts are increased to a greater extent in sicker patients after LDLT, resulting in an increased activation of growth factors and active replication of hepatitis C virus.

Regarding the impact of center experience in performing LDLT, a combination of multiple surgical and non-surgical factors could explain the improved outcomes, as previously reported in the A2ALL study [20, 21]. That study showed a significant improvement in graft outcome after the first 15–20 cases, which was attributed to improvements in patient selection, perioperative management and surgical techniques. However, it should be noted that both A2ALL and non-A2ALL centers in the USA had extensive experience in performing deceased donor liver transplantation before starting LDLT. This differs from the clinical experience in Eastern countries. At our institutes, many surgical and non-surgical refinements have been introduced over the last 15 years [22]. The main surgical refinements include recipient high hilar dissection [23], controlling portal hypertension by splenectomy [24] and aggressive reconstruction of the middle hepatic vein tributaries [25]. Non-surgical refinements include three-dimensional anatomical and volumetric analysis [26], recipient risk evaluation [27] and the application of early enteral nutrition [28].

The managing strategies for recurrent hepatitis C have also been changed with increasing clinical experience. It has long been difficult to differentiate between acute rejection and early recurrent hepatitis C, and bolus doses of steroids were used to prevent possible rejection, resulting in the development of aggressive hepatitis C, as in other centers [29]. Currently, we treat patients with hepatitis C with a higher but more stable immunosuppression regimen to avoid acute rejection, which require bolus steroids for treatment. The incidence of acute rejection following bolus steroid administration has decreased significantly since Era-II (9/119 vs. 5/238 in Era-I, $p = 0.012$). This was largely due to the administration of interferon, which allowed for higher rates of biochemical and viral responses [14].

The relationship between PVP and the presence of major shunt vessels seems to be mutually related. Advanced liver disease causes an increased PVP, resulting in the creation of major shunts, which then reciprocally decrease the PVP. Moreover, the PVP after reperfusion is determined by the graft compliance, PV inflow and the regenerative activity of the graft [9]. Therefore, we believe that the development of major shunt vessels is one of the significant factors reflecting the hepatic disease severity, and thus the MELD scores [22]. The current results showing the significance of major shunt vessels implied that a deteriorated recipient condition had a significant impact on the short-term graft outcomes. However, the PVP had no significant impact in the current series, possibly because a higher PVP was intentionally controlled by splenectomy [13]. A lack of PVP modulation might have resulted in a finding that the PVP was a significant indicator for inferior graft survival.

The significant weakness of this study might be the learning curve bias. Since 2004, we have introduced many surgical and non-surgical refinements in LDLT, including splenectomy for high PVP [13], the introduction of a vessel sealing system [13], aggressive reconstruction of the middle hepatic tributaries in right lobe LDLT [25], the introduction of early enteral nutrition for preventing septic complication [28] and tailored antiviral treatment for recurrent hepatitis C [14]. However, our data showed that the accumulation of experiences significantly improved the outcomes in difficult cases.

In conclusion, the graft outcomes in patients with high MELD scores and the presence of hepatitis C were particularly poor. In patients with these risk factors, LDLT should be performed at experienced centers and/or by experienced surgeons.

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Conflict of interest No financial or other conflict of interest exists with the authors.

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Obstructing Spontaneous Major Shunt Vessels Might Not Be Mandatory to Maintain Adequate Portal Inflow in Living Donor Liver Transplantation

We read with great interest the article by Ikegami et al. (*1*), who reported on the necessity of obstructing spontaneous major shunt vessels in living donor liver transplantation (LDLT). Since 2000, all identified major (≥10 mm) portosystemic shunt vessels have been ligated during LDLT to maintain an adequate portal inflow. Good outcomes in managing portal vein (PV) hemodynamics in LDLT support this concept. However, regardless of the size of the portosystemic shunt, we do not always obliterate them during LDLT if there is sufficient portal flow into the graft after reperfusion.

Since 1997, we have performed 187 LDLTs in our hospital. Because we have digital data of imaging studies on computed medical chart, which made it possible for us to measure the diameter of the vessels accurately beginning in 2005, 137 LDLT cases were available for retrospective analysis. Of these, 45 patients had major spontaneous shunt vessels (diameter, ≥10 mm on computed tomography). Of these 45 patients, 8 underwent intraoperative ligation of spontaneous shunt vessels, and 1 was excluded from the analysis because the patient underwent anastomosis between the collateral shunt vessel and the PV. Of the 36 unligated patients, 8 were postoperatively complicated: 2 with portosystemic encephalopathy, 1 with decreased PV flow and increased ammonia, 2 with PV thrombosis, 2 with stenosis of PV anastomosis, and 1 with decreased PV flow. Unfortunately, 1 patient died at postoperative day 67 because of decreased PV flow with subsequent graft dysfunction. Another 7 patients were treated as follows: 1 relaparotomy due to PV thrombosis; 3 effective balloon-occluded retrograde transvenous obliterations (BRTOs) for 2 patients with hepatic encephalopathy and 1 with decreased PV flow and increased ammonia; 2 angiographies with stent placement for patients with stenosis of the PV anastomosis; and 1 retrans plantation due to PV thrombosis with subsequent liver failure.

Of our 36 unligated patients, 27 experienced no complications because of major shunt vessels after LDLT. Therefore, we believe that it is not always necessary to expose the patient to additional risk because of the ligation of major shunt vessels during LDLT, if there is sufficient portal flow into the graft after reperfusion. Despite new technical approaches, ligation is not always easy and sometimes even still dangerous, especially for patients who have previously undergone the several abdominal surgeries that have likely led to the formation of severe intra-abdominal adhesions. It should also be noted that even after shunt vessel ligation during LDLT, there is still a chance of recurrence after surgery, and this procedure might be ineffective (*2*).

It remains controversial whether a portosystemic shunt detected before liver transplantation should be occluded during liver transplantation. A portosystemic shunt could decrease PV flow after liver transplantation, leading to the subsequent formation of PV thrombosis, graft dysfunction, and/or other serious consequences (*3, 4*). On the other hand, the presence of a shunt can have a positive effect on liver perfusion in cases with relative portal hypertension in the early postoperative period, especially after LDLT (*3, 5*).

As Ikegami et al. also described in their study, BRTO has recently been reported to be a less invasive treatment for portosystemic shunt complications after LDLT (*6, 7*). The effectiveness of BRTO treatment for patients after LDLT with gastric varices and liver dysfunction, including hyperbilirubinemia and/or hyperammonemia, and without hepatic encephalopathy caused by prolonged portosystemic shunts has also been reported (*7*). One patient analyzed in the present study was complicated with decreased PV flow and high ammonia, and underwent BRTO for a splenorenal shunt at day 6 after LDLT. BRTO therefore seems to be effective, regardless of the interval between the development of complications due to the portosystemic shunt and LDLT. Even if the complication occurs because of a major shunt vessel after LDLT, it can be managed with a less invasive treatment strategy.

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Obstructing Spontaneous Major Shunt Vessels Is or Might Not Be Mandatory in Living Donor Liver Transplantation: The Authors' Reply

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We much appreciate Dr. Takashuki and colleagues' comments on our study. As we have reported, our strategy in the management of portal hemodynamics in LDLT is normalizing portal hemodynamics by ligation of major shunts to treat portal stealing combined with splenectomy to treat portal over inflow (*1*). On the other hand, Nagasaki group commented that major shunt vessels should not necessarily been obstructed because shunt ligation is not always easy and might be a chance to cause over portal inflow resulting in graft dysfunction. Although indication of ligation of major shunt vessels was not described in the letter, the Nagasaki group performed shunt ligation for 8 (17.8%) cases among 45 LDLTs with major shunt vessels. Among the cases without shunt ligation (n=37), 8 (21.6%) patients had portal complications including retransplantation (n=1) and graft loss due to decreased portal flow (n=1).

The difficulties in managing portal hemodynamics after LDLT include changing portal pressure caused by graft regeneration, available approaches for delayed shunt occlusion, and possible graft dysfunction associated with excessive portal inflow by shunt occlusion. Portal pressure after LDLT is primarily determined by graft regeneration but also influenced by other multiple factors including graft quality, graft size, and surgical and nonsurgical complications (*2*). A patient with large shunts always has risk of deterioration, especially if a transplanted graft has unfavorable factors for compliance including older donor age, smaller graft size, and jeopardized venous outflow. On the other hand, if a transplanted graft has favorable factors for better compliance including larger graft size, it could be away from portal stealing even under the presence of shunt vessels. However, even in such cases, nonsurgical complications including acute rejection could cause portal stealing with graft dysfunction. Thus, obstruction of major shunts is a great insurance for secure portal inflow.

The possible approaches for various types of shunts are important issue. For gastroesophageal shunts, no interventional approach is possible, and the only available approach is surgical approach including our stapling division technique (*3*). For splenorenal shunts, BRTO is applicable, but renal dysfunction caused by the use of iodine contrast medium is issue, especially in early posttransplant periods (*4*). For mesocaval shunts, transvenous approach including BRTO is a good option because such shunts usually has very short communication between portal system and vena cava and BRTO possibly causes portal or pulmonary embolization. Thus, we think that BRTO is an available option only for delayed occlusion of splenorenal shunts.

Graft dysfunction caused by excessive portal inflow is another issue caused by shunt ligation. To overcome the issue, we exclusively perform simultaneous splenectomy while shunt ligation is performed. The combination of removing huge spleen and obstructing major shunts, supply, and drainage of portal flow, respectively, is just the trial for normalizing portal hemodynamics (*1*). Application of these strategies has significantly improved graft functions and survivals after LDLT over time at Kyushu University (*5*). Although we have applied our strategies with mandatory shunt ligation for years with acceptable outcomes, further multicenter studies for identifying indication of mandatory shunt ligation is necessary.

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T.I. participated in conception and design and drafting of the manuscript. T.Y. participated in conception and design. Y.Y. participated in measurement of portal flow and pressure. N.H. participated in collection of clinical data. H.K. participated in posttransplant interventional radiology. K.S. approved the manuscript. Y.M. participated in the final approval of the manuscript.
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Suppression of Autophagy During Liver Regeneration Impairs Energy Charge and Hepatocyte Senescence in Mice

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Autophagy is a homeostatic mechanism that regulates protein and organelle turnover and uses the amino acids from degraded proteins to produce adenosine 5'-triphosphate (ATP). We investigated the activity of autophagy-associated pathways in liver regeneration after partial hepatectomy (PHx) in liver-specific autophagy-related gene 5 (Atg5) knockout (KO) mice. Liver regeneration was severely impaired by 70% PHx, with a reduction in postoperative mitosis, but a compensating increase in hepatocyte size. PHx induced intracellular adenosine triphosphate and β-oxidation reduction as well as injured cellular mitochondria. Furthermore, PHx in Atg5 KO mice enhanced hepatic accumulation of p62 and ubiquitinated proteins. These results indicated that reorganization of intracellular proteins and organelles during autophagy was impaired in the regenerating liver of these mice. Up-regulation of p21 was associated with hepatocyte senescence, senescence-associated β-galactosidase expression, irreversible growth arrest, and secretion of senescence-associated molecules, including interleukin (IL)-6 and IL-8. *Conclusion*: These findings indicate that autophagy plays a critical role in liver regeneration and in the preservation of cellular quality, preventing hepatocytes from becoming fully senescent and hypertrophic. (HEPATOLOGY 2014;60:290-300)

Liver regeneration is a well-orchestrated process, in which complex signaling pathways coordinate the progression of distinct stages, including withdrawal of hepatocytes from quiescence ("priming phase"), cell-cycle entry and progression, cessation of cell division, and return of hepatocytes to quiescence.[1] Although hepatocytes rarely divide under normal circumstances, the liver has a remarkable ability to regenerate after surgical removal or after viral or chemical injury.[1-3] For example, after partial hepatectomy (PHx) of two thirds of the liver, a rodent model of liver regeneration, the remaining third grows rapidly to restore the liver's original mass, structure, and function within a few days. Indeed, >95% of mature hepatocytes synchronously exit the G_0 phase and re-enter the cell cycle.[2,3] Hepatocytes are the first cells to replicate, followed sequentially by biliary epithelial cells, Kupffer cells, stellate cells, and sinusoidal endothelial cells.[1-3] Hepatocyte proliferation in response to cytokines and growth factors plays a central role in liver regeneration.[1-3]

Early stress signals occurring after PHx may be the result of an increase in energy demand per unit liver mass. Remnant tissue retains liver-specific functions, such as gluconeogenesis and ureagenesis, and continues to produce adenosine 5'-triphosphate (ATP) for synthesis of proteins, nucleic acids, and other cell constituents.[4,5] There was a marked decline in ATP within 6 hours after PHx, which was maintained throughout the prereplicative period. Moreover, the change in

Abbreviations: Akt, protein kinase B; ALT, alanine aminotransferase; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; Atg, autophagy-related gene; ATP, adenosine 5'-triphosphate; BrdU, bromodeoxyuridine; CQ, chloroquine; ERK, extracellular signal-regulated kinase; FFAs, free fatty acids; HGF, hepatocyte growth factor; IHC, immunohistochemistry; IL, interleukin; KO, knockout; LC3, microtubule-associated protein 1 light chain 3; LW/BW, liver weight/body weight ratio; mRNA, messenger RNA; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor kappa B NPCs, nonparenchymal cells; Nrf2, nuclear factor (erythroid-derived 2)-like 2; OS, oxidative stress; PCR, polymerase chain reaction; PHx, partial hepatectomy; SA-β-gal, senescence-associated β-galactosidase; SASP, senescence-associated secretory phenotype; SIRT1, sirtuin-1.

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hepatocytes from the quiescent to replicative mode during the early phase of recovery was accompanied by reorganization of intracellular proteins and organelles.[1-3] However, the mechanisms underlying restoration of normal liver function, including the role of systemic metabolism, and reorganization of intracellular content throughout the replicative period after PHx remain unknown.

Autophagy is a homeostatic mechanism that regulates turnover of long-lived or damaged proteins and organelles, buffers intracellular constituents, and supplies amino acids taken from degradation products of the autolysosome.[6] The first step of autophagy involves the formation of a lipid bilayer structure, which sequesters cytoplasmic materials to form autophagosomes. These autophagosomes engulf organelles and then fuse with lysosomes to form mature autolysosomes, in which the sequestered proteins are digested into amino acids by lysosomal enzymes.[7] Based on its ability to reorganize intracellular proteins and organelles, and to modulate intracellular energy,[6-8] autophagy may be essential for liver regeneration, and its activities may be critical for mitotic or hypertrophic hepatocytes.

In a mouse model with liver-specific knockout (KO) of autophagy-related gene 7, livers exposed to long-term surveillance exhibited significant hepatomegaly with aggregates of unfolded proteins,[9] which is apparently inconsistent with the role of autophagy in promoting cell proliferation. To investigate the mechanism by which autophagy regulates hepatocyte proliferation and liver growth, we developed a mouse model of liver regeneration with liver-specific KO of autophagy-related gene 5 (L-Atg5 KO mice). Unexpectedly, liver regeneration after PHx was significantly delayed and was accompanied by delays in DNA synthesis and cell-cycle arrest during liver regeneration. Furthermore, we found that impaired hepatocyte proliferation was biologically distinct from cell-cycle arrest because it represented cellular senescence accompanied by increased cell size (hypertrophy) and accumulation of senescence-associated enzyme β-galactosidase (SA-β-gal).[10] These results suggest that autophagy plays critical roles in liver regeneration after PHx by maintaining intracellular energy production, as well as constitutive proteins and organelles, to prevent hepatocyte senescence.

Materials and Methods

Generation of Liver-Specific Atg5-Deficient Mice. $Atg5^{flox/flox}$; Mx1-Cre mice were generated by crossing $Atg5^{flox/flox}$ mice, in which exon 3 of the Atg5 gene is flanked by two loxP sequences,[11] with transgenic mice expressing Cre recombinase under control of an Mx promoter (Mx-Cre).[12] Both strains of mice were purchased from The Jackson Laboratory (Bar Harbor, ME).[9] Recombination was successful in >90% of all hepatocytes from $Atg5^{flox/flox}$; Mx-Cre mice. Mice were genotyped by polymerase chain reaction (PCR) to detect wild-type Atg5 and $Atg5^{flox}$ alleles, as previously described.[11] Cre expression in livers of $Atg5^{flox/flox}$; Mx1-Cre mice was induced by intraperitoneal injection of 300 μL polyinosinic acid/polycytidylic acid (Sigma-Aldrich, St. Louis, MO) at a concentration of 1 mg/mL in water, three times at 48-hour intervals. Mice were maintained in a room with alternating 12-hour light/dark cycles. Animals received humane care in compliance with the institutional guidelines of the Graduate School of Medical Sciences, Kyushu University (Fukuoka, Japan). The study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Animal Studies. PHx was performed in male and female mice 6 weeks of age. Mice were anesthetized with ether and subjected to approximately 70% PHx by removing the left lateral and median lobes, after midventral laparotomy.[2,13] The mortality rate after 70% PHx was <1%.

Plasmids. A plasmid containing an inactive mutant of Atg4B ($Atg4B^{C74A}$), a protease that processes pro-LC3 (microtubule-associated protein 1 light chain 3) paralogs and hampers conversion of LC3-I to LC3-II, was a kind gift from Dr. Yoshimori and was prepared as previously described.[14]

For materials and methods in more detail, see the Supporting Information.

Results

Generation of L-Atg5 KO Mice. PCR analyses showed that the subset of autophagic genes was expressed in livers of L-Atg5 KO mice ($Atg5^{flox/flox}$; Mx-Cre mice; Supporting Fig. 1A). Livers of L-Atg5

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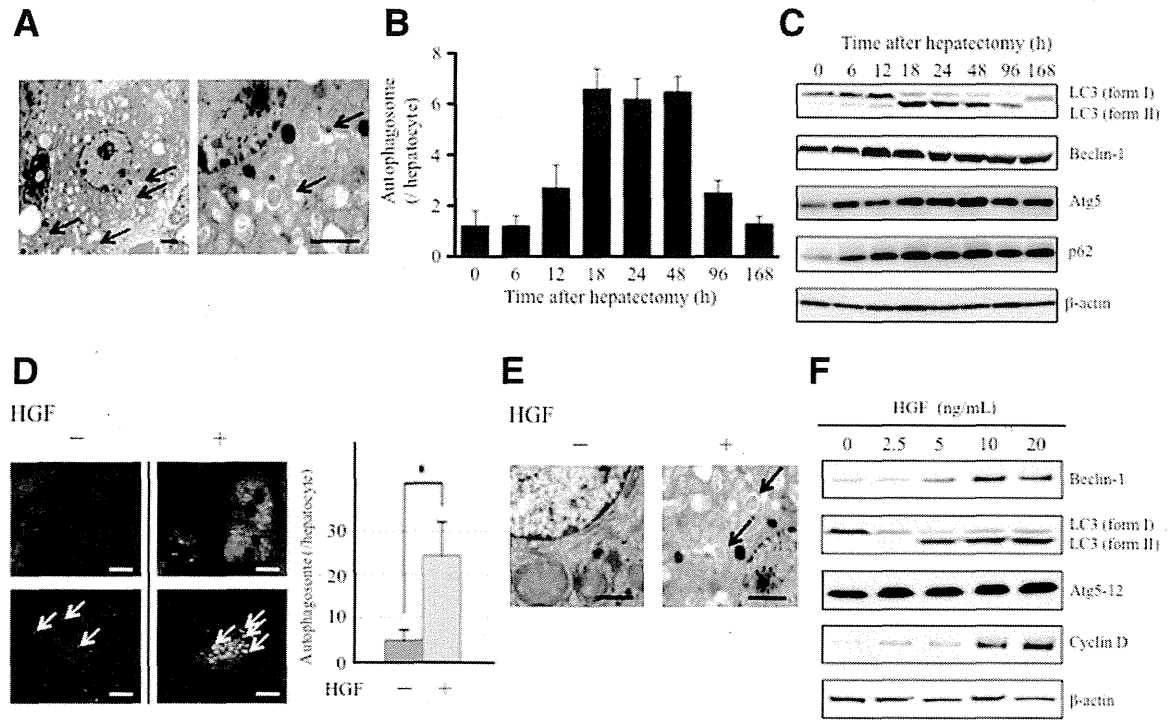

Fig. 1. Effects of PHx on autophagic activity in the regenerating liver and in primary cultured hepatocytes. (A) Autophagosomes in the regenerating liver. Arrows, autophagosomes. Scale bar, 1 μm. (B) Number of autophagosomes in hepatocytes at the indicated times after hepatectomy. (C) Expression of the autophagy-related genes, LC3-I, LC3-II, Beclin-1, Atg5, and p62, in control mice at 0-196 hours after 70% PHx (representative western blottings are shown). (D) Immunofluorescent analysis of LC3 in primary cultured hepatocytes in response to HGF stimulation. Arrows, autophagosomes. Scale bar, 10 μm. Number of autophagosomes in hepatocytes treated without or with HGF by immunofluorescent analysis of LC3. (E) Electron microscopic images of autophagosomes (arrows) in hepatocytes treated without or with HGF. Scale bar, 1 μm. (F) Effects of HGF on expression levels of LC3-I, LC3-II, Beclin-1, Atg5-12, and cyclin D in primary cultured hepatocytes (representative western blottings are shown). $*P < 0.05$.

KO mice displayed appropriate responses to 24-hour starvation with increased expression of Beclin-1 and LC3, similar to control mice, but lacked Atg5 expression. Structure and morphology of the liver (Supporting Fig. 1B) and other organs, including the spleen, heart, lung, kidney, and brain (Supporting Fig. 2), were normal. In terms of liver injury, serum alanine aminotransferase (ALT) levels were higher in L-Atg5 KO mice at 21 days after injection than in control mice, consistent with a previous report[9] (Supporting Fig. 3). At 8 weeks of age, liver and body weights of L-Atg5 KO mice were slightly lower than those of control mice (Supporting Fig. 4), consistent with previous results using $Atg5^{flox/flox}$; nestin-Cre mice.[11]

Autophagic Activation in the Regenerating Liver After PHx. In control mice, 70% PHx caused an increase in the number of autophagosomes, peaking at 18 hours after PHx, which was maintained until 48 hours, but returned to baseline after 4 days (Fig. 1A,B). Expression of p62, which regulates ubiquitin-positive protein aggregates during autophagic deficiency, remained elevated for the entire study period (Fig. 1C). *In vitro*, isolated hepatocytes showed marked dose-dependent autophagy in response to hepatocyte growth factor (HGF; Fig. 1D-F). Knockdown of autophagy using a lentivirus vector encoding mutant Atg4B, which is indispensable for lipidation of LC3 proteins, reduced HGF-stimulated autophagy (Supporting Fig. 5). HGF treatment (5-20 ng/mL) increased levels of phosphorylated protein kinase B (Akt; Thr308 and Ser473) and phosphorylated mammalian target of rapamycin (mTOR)1 (Supporting Fig. 6). HGF also increased LC3-II levels (Fig. 1F), which are usually down-regulated by phosphorylated mTOR. This finding may be the result of increased phosphorylated adenosine-monophosphate (AMP)-activated protein kinase (AMPK) levels in HGF-treated proliferative hepatocytes (Supporting Fig. 6).

In the LC3 turnover assay, LC3-II levels were increased by treatment with chloroquine (CQ), even before 70% PHx. However, differences in LC3-II levels in the presence and absence of CQ were greater at 24 and 96 hours after 70% PHx, compared with before 70% PHX (Supporting Fig. 7A). Levels of p62, which is normally degraded during autophagy, were increased by CQ, especially after 70% PHx