Lack of grafted liver rejuvenation in adult-to-pediatric liver transplantation

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Key words: living donor liver transplantation, Senescence Marker Protein-30, rejuvenation, pediatric, graft

Abstract

Background: A grafted donor liver should grow and survive under the different conditions presented by a liver transplantation recipient. It has remained unclear, however, whether the age of a grafted liver can be modulated by recipient factors.

Aims: This study investigated whether a grafted aged donor liver can be rejuvenated in a pediatric recipient.

Methods: Of 119 living donor liver transplants, 10 pairs have been adult-to-pediatric combinations. Senescence Marker Protein-30 (SMP30), which is a protein that is remarkably reduced upon aging, was used as a senescence marker. Immunohistochemical staining for SMP30 was performed in biopsy specimen after LDLT. Re-expression of SMP30 was investigated in a biopsied adult liver (n=6) that had been transplanted in a pediatric recipient.

Results: A remarkable expression of SMP30 was seen in a control pediatric normal liver in comparison with that in an aged adult donor biopsy. Re-expression or an increase in SMP-30 was not observed in the liver of any pediatric recipient who had received an adult liver.

Conclusion: An adult grafted liver does not appear to rejuvenate in a pediatric recipient.

Introduction

Many investigators have reported worse outcomes of orthotopic liver transplantation (OLT) with aged donor liver graft [1)2)]. Especially since living donor liver transplantation (LDLT) is performed with a partial liver graft, lower patient survival has been reported with senile donor grafts than with graft from younger donors [3)]. However, if grafted aged liver could adapt to the environment of young or pediatric recipients, resulting in rejuvenation, an aged grafted liver could be regarded as a young graft through the life of the recipient as observed in vitro experiment [4)]. However, no report of rejuvenation of graft liver in humans has been made thus far.

Senescence Marker Protein-30 (SMP30) was found in the liver of rats and is reported to markedly decrease through the senescence process [5, 6)]. SMP-30 is associated with Vitamin C synthesis [7)], and it has been reported that the senescence process proceeds four times faster in SMP-30 knock-out mouse than in normal mouse [8)]. Thus SMP30 is one of the key elements of senescence. It has also been reported that when SMP30 decreases, resistance to infectious pathogens would decrease along with organ function, resulting in aging of the organs [9-11)].

We therefore used this unique protein expression in the liver to clarify whether a grafted liver can successfully become rejuvenated in adult-to-pediatric LDLT.

Patients and Methods

Of 119 living donor liver transplants up until March 2010, 10 pediatric liver transplantations were performed with grafts obtained from parents of the recipients. Eleven biopsies were performed in the 6 patients, when it was clinically indicated. Afterwards, re-expression of SMP30 was investigated in a biopsied adult liver (n=6) that had been transplanted in a pediatric recipient. Characteristics of recipients and donors are described in Table 1.

Methods of LDLT

LDLT methods have been reported elsewhere[12]. All partial liver grafts were preserved in University of Wisconsin solution and implanted using a piggy-back technique. A dual or triple immunosuppressive regimen was used that included

tacrolimus or cyclosporine A, steroid, and mycophenolate mofetil. Biopsy-proven rejections were treated if clinical and laboratory signs mandated steroid bolus treatment.

Immunohistochemical staining for SMP30

Four-micrometer liver sections were deparaffinized and rehydrated through 100%, 95%, and 90% ethanol. In terms of the heat-induced antigen retrieval protocol, a 40-minute treatment with Target Retrieval Solution (code S2031, DAKO, Carpinteria, CA) was followed by a 20-minute cool-down period at room temperature. The tissue sections were then immunostained using an automated staining system (Autostainer Plus, DAKO, Carpinteria, CA). Slides were incubated with an anti-SMP30 polyclonal antibody (1:80 dilution, code SML-ROI001,SHIMA Laboratories) for 30 minutes at room temperature and subsequently with Histofine Simple Stain MAX-PO (MULTI) (Nichirei, Japan). Incubation was performed overnight at 4 degrees Celsius, followed by a wash in three changes of PBS for 5 minutes. For all staining, the reaction product was developed with the use of 3-diaminobenzidine tetra hydrochloride and H_2O_2. The sections were counterstained with Meyer hematoxylin-Eosin. Visualization was labeled polymer (EnVision+ system; code K4001,Dako) for 30 minutes at room temperature,

3,3′-diaminobenzidine as a chromogen for 5 minutes, and hematoxylin as a counterstain for 5 minutes.

Among stained hepatocytes, semiquantification was performed based on the comparison with positive controls and negative controls. Positivity of staining was classified as follows; negative-: weak+/-: moderate +; strong ++.

Results

Remarkable expression of SMP30 was seen in a control pediatric liver (10 years old, male, normal liver obtained at liver resection for hepatoblastoma, Fig. 1A, B) and 2 other pediatric patients (3 years old, patient with neuroblastoma; 1 year old, patient with hepatoblastoma). On the other hand, very limited expression of SMP30 was observed in a case of senile liver (64 years old, male, living donor, Fig. 1C, D), which can be regarded as a negative control.

Fig. 2A depicts diseased liver explanted for liver transplantation in a 1-year-old female (Case 2). Even in a diseased liver due to biliary atresia, SMP30 was expressed (Fig. 2A, B). At the time of liver transplantation, the graft liver did not express SMP30 (37 years old, mother, Fig. 3). After the graft liver was transplanted into the recipient, a

series of liver biopsies was performed. Four and 5 years after the LDLT, no increase in SMP30 in the liver was observed (Fig. 3A-D). Similarly, in the other pediatric recipient for LDLT, SMP30 was not increased over time (Fig. 4A,B: 40 years old living donor, Fig. 4C,D: 5 years after the LDLT, Case 4).

The results of the immunohistochemical staining for SMP30 are summarized in Table 1. No re-expression of SMP30 was observed in any of the cases.

Discussion

In this study, we clearly demonstrated no re-expression of a senescent marker, SMP30 in aged liver graft transplanted in pediatric patients. This is the first literature report indicating that no rejuvenation of a graft occurs after liver transplantation based on SMP30.

With regard to the relationship between aging and liver function, it has been reported that liver function is not altered in the aging process [13], while Hyams has reported a slight derangement of liver function in aged patients [14]. Some investigators have also reported that with aging, drug metabolism of propranolol decreases in

hepatocytes of the liver based on an in vitro experiment [15]. On the other hands, a relationship between aging and liver regeneration has been reported in vitro, with DNA synthesis in the hepatocytes of aged rats decreasing but with a preserved repair process as compared with that seen in young rats [16]. As to in vivo experiments, there have been some reports indicating a decrease or delay (24 hours) in DNA synthesis after partial hepatectomy in elderly rats [17,18]. Tsukamoto et al. have reported a 24-hour delay in liver regeneration with aged, 60-week-old rats compared with 6-week-old rats [19] .

In the field of liver transplantation, rejuvenation of the liver has not been fully investigated. Sakai et al. have reported that the survival rate after OLT is similar between aged (28 months old) and young rats (5 months old) [20]. However, it has been revealed that fibrosis, bile duct proliferation, and pigment deposition are more observed in aged grafts than in young rats, implying that the liver function of aged liver grafts could be bearable but that the aging process is indeed advancing. Recently, Selzner et al. have reported that in the rat model with ischemia reperfusion injury, apoptosis induced by TNF-α is increased in aged rats compared with young rats [21]. This mechanism might be one of the causes of poor graft function in an aged liver graft.

On the other hand, Conboy et al. have reported the rejuvenation of aged progenitor cells in response to exposure to a young systematic environment [4]. In this experiment, it was also reported that old hepatocytes can be rejuvenated in a serum of young rats in terms of DNA synthesis and Ki67 expression, suggesting the possible rejuvenation of hepatocytes.

With regard to hepatocyte proliferation, SMP30 has a suppressive effect on cell proliferation in the SMP30 knock-out mouse model [22]. However, many reports have indicated that SMP30 overexpression can decrease reactive oxygen species and exert a cytoprotective effect [23]. In addition, the possible involvement of apoptosis reduction has been reported [24]. Other than the liver, decreases in SMP30 are related to organ dysfunction such as that of the brain, ear, and lung [25-27]. If SMP30 expression is increased in grafted aged liver, the graft survival rate after OLT with aged donor liver could be as good as that with young donor liver.

SMP30 was used as a marker for senescence in the present study because it is ubiquitously expressed in pediatric liver and is stable for immunohistochemical investigations of paraffin-fixed samples. Another possible marker of senescence is the

telomere length, although a huge number of samples is needed to determine whether there are differences in telomere length, making this method impractical.

In conclusion, it was demonstrated in humans that rejuvenation, as assessed by SMP30, was not observed in the setting of adult-to-pediatric liver transplantation.

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Figure Legends

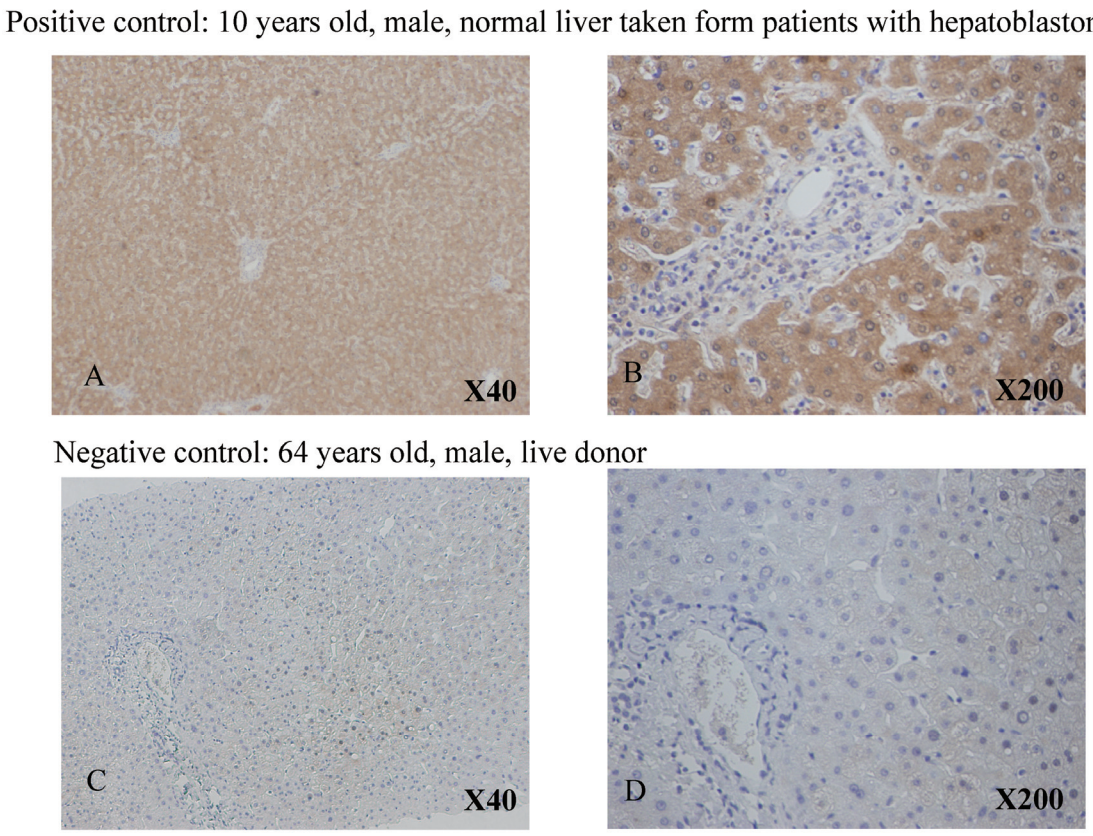

Fig. 1: Immunohistochemical staining for SMP30

A (x40), B (X200): Positive control: 10 years old, male, normal liver taken form patients with hepatoblastoma.

C (x40), D (x200): Negative control: 64 years old, male, live donor

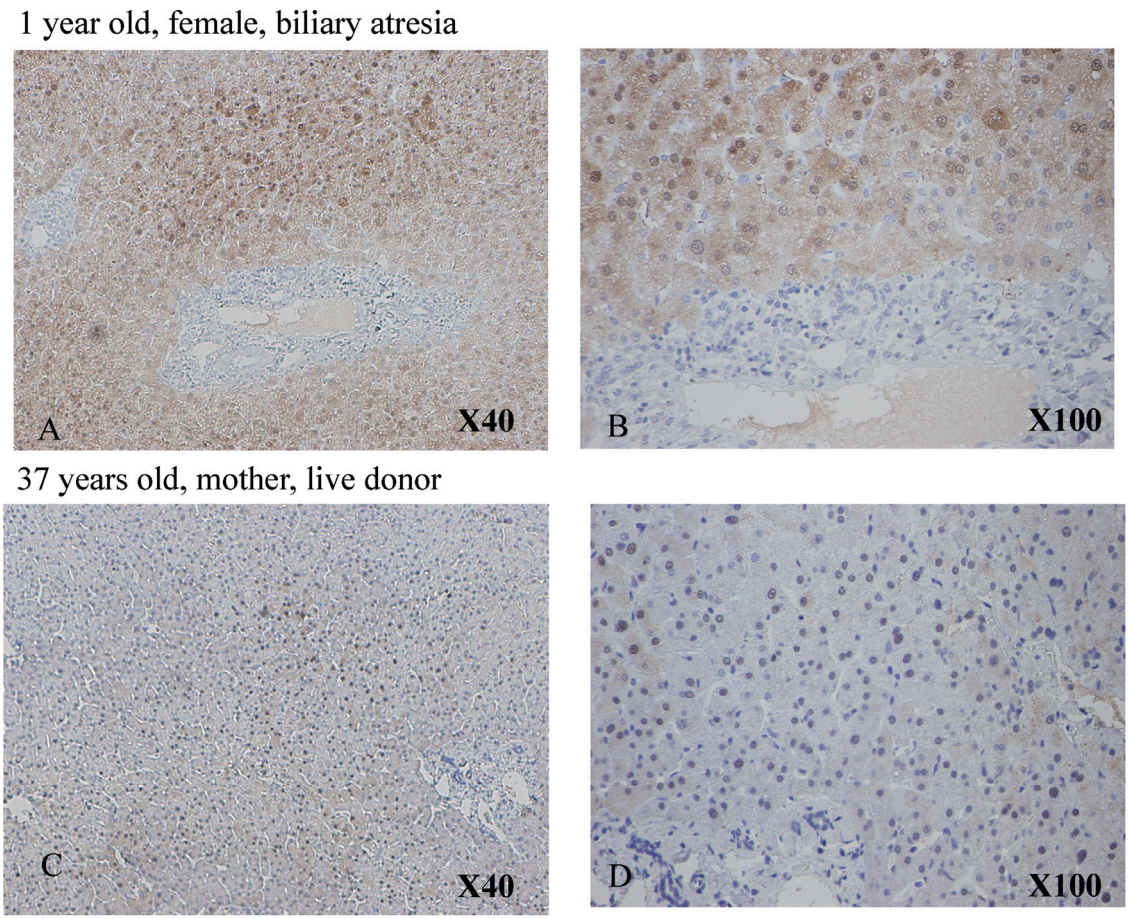

Fig. 2: Immunohistochemical staining for SMP30 (Case 2)

A (x40), B (X100): 1 year old, female, biliary atrasia

C (x40), D (x100): 37 years old, mother, live donor

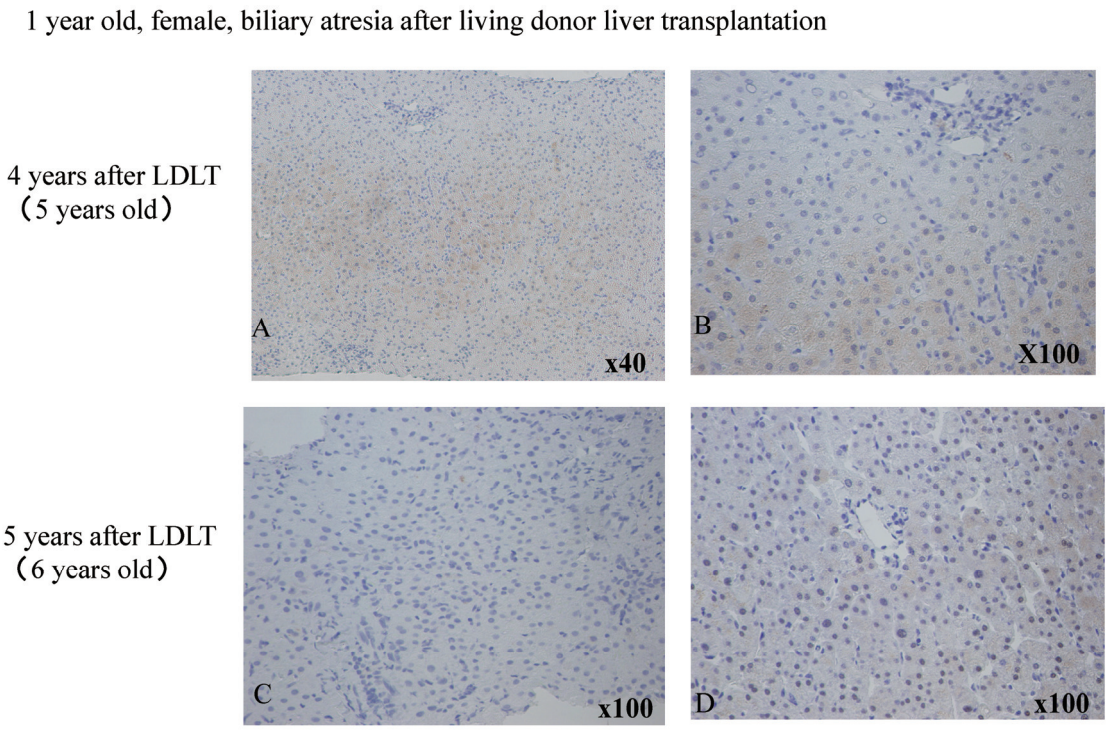

Fig. 3: Immunohistochemical staining for SMP30 (Case 2)

1 year old, female, biliary atrasia after living donor liver transplantation

A (x40), B (X100): 4 years after LDLT（5 years old）

C (x100), D (x100): 5 years after LDLT （6 years old）

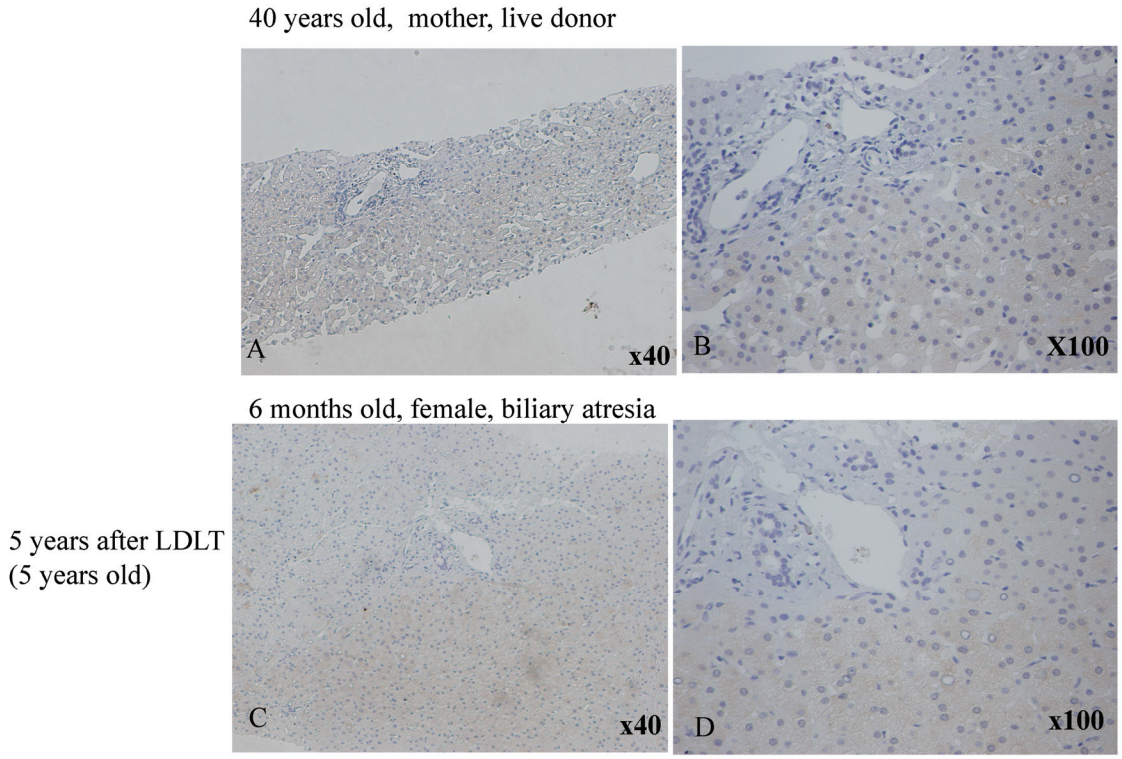

Fig. 4: Immunohistochemical staining for SMP30 (Case 4)

A (x40), B (X100): 40 years old, mother, live donor

C (x40), D (x100): 6 months old, female, biliary atrasia (5 years after LDLT)

Table 1. Immunohistochemical staining for SMP-30

	Donor		Recipient		
No	Age/Relation	SMP-30	Age /Primary disease	Liver biopsy (time after LDLT)	SMP-30
1	40/Mother	-	6/Biliary atresia	2M	-
				2Y	-
				8Y	-
2	40/Mother	+	1/Biliary atresia	Explant	+
				4Y	+
				5Y	+/-
3	37/Mother	-	5/Biliary atresia	7Y	-
				8Y	-
4	40/Mother	+	11M/Biliary atresia	Explant	+
				5Y	+/-
5	37/Mother	-	7M/Fulminant hepatic failure	2Y	-
				3Y	-
6	35/Father	-	10M/Biliary atresia	0.5Y	-

		SMP-30
Controls	10Y/Hepatoblastoma (Normal liver)	++
	3Y/Neuroblastoma (Normal liver)	++
	1Y/Hepatoblastoma (Normal liver)	++