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Improved Hepatic Regeneration With Reduced Injury by Redox Factor-1 in a Rat Small-Sized Liver Transplant Model

Lei Guo[a], Sanae Haga[b], Shin Enosawa[b], Katsutoshi Naruse[a], Yasushi Harihara[a], Yasuhiko Sugawara[a], Kaikobad Irani[c], Masatoshi Makuuchi[a] and Michitaka Ozaki[b,d,*]

[a] *Department of Artificial Organ and Transplantation Surgery, Graduate School of Medicine and Faculty of Medicine, University of Tokyo, Tokyo, Japan*
[b] *Bioengineering Laboratory, Department of Innovative Surgery, National Research Institute for Child Health and Development, Tokyo, Japan*
[c] *Division of Cardiology, Department of Medicine, The John Hopkins University School of Medicine, Baltimore, MD, USA*
[d] *Department of Food and Health Science, Okayama University Graduate School of Medicine and Dentistry, Faculty of Medicine, Okayama, Japan*
**Corresponding author: Michitaka Ozaki, mozaki@nch.go.jp*

Redox factor-1 (Ref-1) has been shown to function in a redox-dependent manner in the cell. This study was designed to examine the effects of Ref-1 on liver regeneration as well as protection against postischemic injury in a rat model of 20% partial liver transplantation. Adenovirus carrying the full length of Ref-1 gene was introduced into liver grafts by *ex vivo* perfusion via the portal vein during preservation. Liver graft weights were assessed, as well as graft histology, serum levels of alanine aminotransferase (ALT)/bilirubin, DNA binding activities of AP-1 and Stat3. Redox factor-1 successfully expressed in the liver graft, improved regeneration by promoting cell proliferation. Overexpression of Ref-1 protein also reduced post-transplant injury and inflammatory reactions in the grafts. The increased serum levels of ALT and bilirubin observed after transplantation were significantly reduced by Ref-1 overexpression. Furthermore, adenovirally overexpressed Ref-1 in mouse liver successfully promoted liver regeneration after simple partial hepatectomy. Interestingly, Ref-1 significantly increased DNA binding of Stat3, but not AP-1. Overexpressed Ref-1 effectively promoted graft regeneration and reduced postischemic injury in a small-sized liver transplantation model. The results of the present study may open a new avenue to clinical transplantation of disproportionately sized grafts in living-related liver transplantation.

Key words: Ischemia-reperfusion injury, redox factor-1 (Ref-1), regeneration, small-size liver transplant, Stat3

Abbreviations: Ref-1, Redox factor-1; AdLacZ, adenovirus encoding inert bacterial LacZ gene; AdRef-1, adenovirus encoding Redox factor-1 gene; PLTx, partial liver transplant; EMSA, electrophoretic mobility shift assay; LDLT, living donor liver transplantation; Stat3, signal transducer and activator of transcription-3; AP-1, activator protein-1.

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Introduction

Since the first successful living-related liver transplantation in an adult patient was reported by Hashikura and colleagues (1), the number of adult-to-adult living-related liver transplants has steadily increased, so that this modality has become established as a standard surgical method for the treatment of late-stage liver diseases (2). Currently, for the sake of donor safety in living donor-liver transplantation (LDLT), it is the left lobe which is most frequently grafted. However, size mismatch of graft and recipient remains a major obstacle to successful LDLT in larger-sized recipients.

Oxidative stress is recognized as a critical cause of injury in reperfused tissue (3), and remains a major concern in liver transplantation. Accumulated data from many studies currently suggest that intracellular as well as extracellular oxidative stress may play an important role in inducing postischemic tissue injury (4–7). At a very early phase of reperfusion, production of reactive oxygen species (ROS) associated with Rac1 has recently been reported to mediate intracellular oxidative stress (8), which consequently activates redox-dependent pathologic signals including NF-κB (9–11). Rac1-mediated ROS generation in the postischemic tissue seems to be intimately involved in postischemic tissue injury, because inhibition of Rac1 by overexpressing its dominant negative form results in suppression of hepatic ROS generation and dramatic improvement of postischemic injury in the mouse liver (11,12).

Therefore, regulating the cellular redox state by suppressing cellular ROS in the postischemic tissue is likely to be crucial for protection against ischemia/reperfusion (I/R)-induced liver injury.

Redox factor-1 (Ref-1), a 37-kDa nuclear protein, was initially described as a key endonuclease involved in base excision repair pathways of a variety of DNA lesions (13). In addition to this function, Ref-1 is known to act as a redox-dependent regulator of various transcription factors, such as AP-1 and NF-κB (14–17), thereby affecting transcriptional regulation of their target genes. Expression of Ref-1 *in vivo* is inversely correlated with susceptibility to postischemic injury in neurons (18). Ozaki et al. reported that adenoviral overexpression of Ref-1 in hepatic tissue results in significant suppression of reperfusion-induced oxidative stress, NF-κB activation, apoptosis, and acute hepatic injury (11). Recently, it was reported that constitutive activation of Stat3 protects against Fas-induced liver injury partly by up-regulation of Ref-1 in a redox-dependent manner (19). This leads to the idea that Ref-1 may function as a redox-dependent signal regulator protecting against oxidant-induced apoptotic cell death. In addition, Ref-1 is also known to activate the serine/threonine kinase, Akt (PKB), which is important for cell survival and cell growth (20–23). Akt phosphorylates various pro-survival molecules, including Bcl-2, $p70^{S6K}$, mTOR, GSK-3, thereby affecting their functions (24). A unique mechanism for the antioxidative and antiapoptotic properties of Akt was recently reported, whereby this kinase phosphorylates and inactivates Rac1, and reduces Rac1-mediated ROS production in reoxygenated hepatocytes, preventing against hypoxia/reoxygenation-induced apoptosis (25). These findings suggest the possibility that Ref-1 effectively protects the liver from post-transplant I/R injury as well as promotes liver regeneration. The multifunctional activities of Ref-1, such as DNA repair activity, regulation of gene expression and redox-dependent signal regulation, make it a prime candidate for gene/protein therapy in various clinical settings.

The present study was designed to investigate the effects of overexpressed Ref-1 on post-transplant injury and regeneration after *ex vivo* adenoviral gene transfer to small-size liver grafts. Here, we describe successful *ex vivo* gene transfer to 20% liver grafts during the cold preservation period, and report that Ref-1 prevents I/R-induced liver injury, and in addition promotes regeneration of small grafts in a rat orthotopic liver transplant model. The underlying mechanism of these effects may involve at least the up-regulation of Stat3 activity in the postischemic liver tissue. However, the precise mechanism remains to be clarified.

Materials and Methods

Animal models and gene delivery

A replication-deficient adenovirus encoding full-length Ref-1 (AdRef-1) was constructed by homologous recombination in HEK 293 cells as previously described (11). A control virus encoding the inert bacterial LacZ gene (AdLacZ) has been previously reported (11). All viruses were amplified in HEK 293 cells, purified on double cesium gradients, and plaque-titered.

Rat partial hepatectomy and *ex vivo* gene transfer (Figure 1A) was performed as follows. Adult male Lewis rats weighing 230–250 g were used as donors and recipients. In an initial experiment, the ratio of liver to body weight was estimated in 15 rats. The mean value of total body weights was 241 ± 4.2 g, and the mean values of liver weights was 10.4 ± 0.3 g. Partial hepatectomy was performed with slight modifications as described by Higgins and Anderson (26). Briefly, the rats were subjected to midventral laparotomy and approximately 80% liver resection (left, right lobe and caudate lobe), and the weight of the remaining liver lobes, was recorded (mean 2.3 ± 0.2 g, 23% of whole liver weight). This weight was recorded as 'liver weight 1' at an initial time-point. Recipients were sacrificed at various time-points after transplantation, and the enlarged remaining lobes were totally removed and their weights were recorded as 'liver weight 2'. Subtracting 'liver weight 1' from 'liver weight 2' made it possible to calculate the percentage weight-increase of the liver graft after a specified period of time.

After partial hepatectomy, liver grafts were washed with ice-cold physiological saline (PS), and immediately infused with PS, AdLacZ (1×10^8 pfu) or AdRef-1 (1×10^8 pfu) through the portal vein in a total volume of 0.6 mL. The IVC and portal vein (PV) of the liver graft were then clamped, and were preserved in ice-cold PS for 30 min. Finally, the liver graft was flushed with ice-cold PS to remove intravascular adenovirus in order to avoid transferring gene to the recipient's other organs before transplantation. Sufficient protein expression by this *ex vivo* gene-transfer method was confirmed by infusing AdLacZ to the liver graft as a preliminary experiment (data not shown).

Orthotopic liver transplantation (OLTx) was performed with revascularization but without hepatic artery reconstruction, as previously described by Kamada (27). In brief, the donor liver was flushed via the portal vein with ice-cold PS, and the suprahepatic vena cava was anastomosed by 7–0 continuous sutures. The portal vein and infrahepatic vena cava were anastomosed by the cuff technique. Greater than 95% of the rats survived this operative procedure. At least five rats per each group were sacrificed 1, 3 and 5 days after PLTx.

Mouse simple partial hepatectomy and intravenous adenoviral gene transfer

C57/Black6 mice (male, 20–25 g) were used for simple 2/3 hepatectomy. Anesthesia was induced with an intraperitoneal injection of Nembutal (pentobarbital sodium, 60 mg/100 g BW). After laparotomy, the left and median liver lobes were surgically resected, and adenoviruses (AdLacZ or AdRef-1) or PS were intravenously injected (1×10^9pfu/100 μL/body). The mice were sacrificed 72 h after hepatectomy and adenoviral injection. The liver/body weight ratio was measured.

The animals were maintained under standard conditions and treated according to the Guidelines for the Care and Use of Laboratory Animals of the National Research Institute for Child Health and Development.

Analysis for liver injury and regeneration

For Western blot analysis, 30 μg of whole liver protein extract was separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane. Antibodies against Ref-1, Stat1/3 and phospho-Stat1/3 (Tyr) (Santa Cruz, CA) were used as primary antibodies (2 μg/mL, 1 μg/mL, 1 μg/mL, respectively).

In order to examine mitogenic response after liver transplantation, animals were intravenously injected with bromodeoxyuridine (BrdU, 50 mg/kg; 0.2%

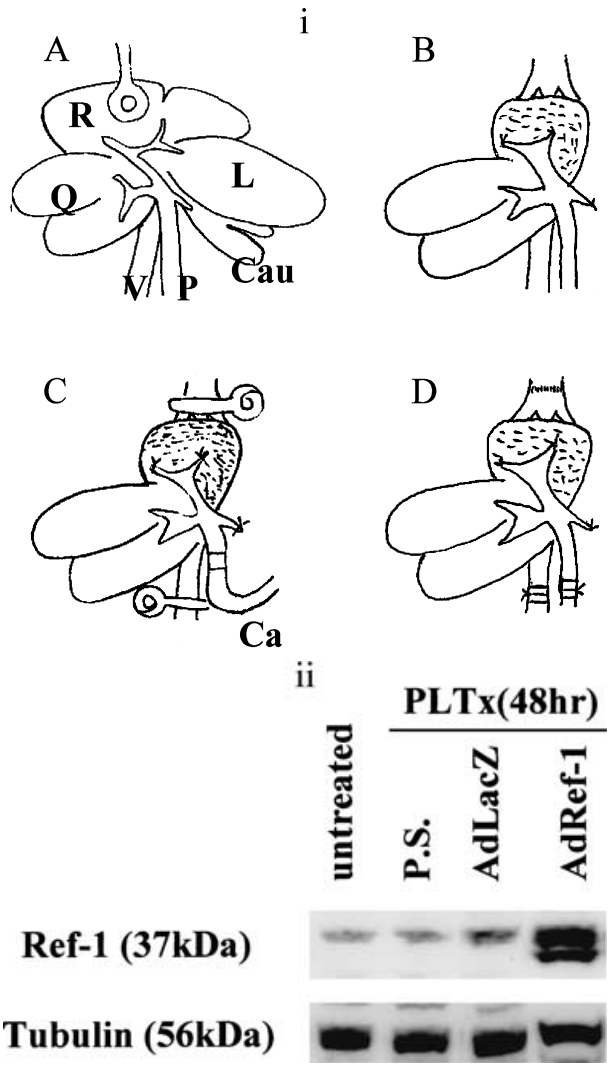

Figure 1: (i) Schema of hepatectomy and *ex vivo* gene transfer. (A) The portal vein and hepatic artery with their branches to the left, right, quadrate and caudate liver lobes are exposed. The hepatic artery and bile duct are not shown. R: right lobe; L: left lobe; Q: quadrate lobe; V: vena cava; P: portal vein; Cau: caudate; and Ca: catheter. (B) 20% liver grafts were harvested by resection of the left, right, and caudate lobes after ligating and dissecting the portal vein, hepatic artery, bile duct collectively and branches of the hepatic vein to each lobe. (C) IVC and the portal vein were clamped and then infused with physiological saline (PS), AdLacZ (1×10^8 pfu) or AdRef-1 (1×10^8 pfu) via the portal vein in a total volume of 0.6 mL in ice-cold PS for 30 min. (D) Orthotopic liver transplantation was performed with revascularization; the suprahepatic vena cava was anastomosed by 7–0 continuous sutures. The portal vein and infrahepatic vena cava were anastomosed by the cuff technique. (ii) Expression of redox factor-1 (Ref-1) protein in hepatic tissue 48 h after partial liver transplant (PLTx). *Ex vivo* transfer of the full-length Ref-1 gene resulted in a significant increase in Ref-1 protein production in hepatic tissue compared with PS, AdLacZ and untreated groups. A slight increase in Ref-1 protein production was observed in the LacZ group compared with the PS and untreated groups. Each immunoblot expresses a representative of at least three independent experiments.

solution in phosphate-buffered saline) 36 h after transplantation, and the liver graft was excised 1 h later. Frozen sections were prepared and stained with an antibody against BrdU (Boehringer Mannheim, Mannheim, Germany). The immunohistochemical study was performed according to the manufacturer's recommendation (Boehringer Mannheim). The number of BrdU-positive nuclei was counted in at least three rats per group, and 3 × 100 hepatocytes were counted for each rat.

Liver grafts were harvested after PLTx. Formalin-fixed, paraffin-embedded specimens were cut into 4-μm-thick sections, and stained with hematoxylin and eosin. Liver specimens were histo-pathologically examined and the number of mitoses was counted to assess hepatocyte proliferation.

Serum was drawn at various time-points after PLTx and used to measure alanine aminotransferase (ALT) and total bilirubin using a standard automatic analyzer (type 7150; Hitachi, Tokyo, Japan).

Electrophoretic mobility shift assay (EMSA)

Nuclear protein extracts from liver tissue were prepared as previously described (2). AP-1 DNA binding was assayed using 5′-CGCTTGATGACTCAGCCGGAA-3′ oligonucleotides as a probe and 5′-CGCTTGATGACTTGGCCGGAA-3′ as a mutant oligonucleotide. Stat DNA binding activity was assayed using SIE m67 oligonucleotides as a probe for Stat3 : 3, Stat1 : 3 and Stat1 : 1 homo-, hetero-dimers (5′-actgGGATTTTTCCCGTAAATGGTC-3′). The nuclear protein extract (5 μg) was reacted with 10^5 cpm of ^{32}P-labeled AP-1- or Stat3-binding consensus oligonucleotides for 30 min in binding buffer (10 mM tris, pH 7.4; 80 mM KCl; 5% glycerol; 1 mM DTT; 0.25 μg dIdC) at room temperature. The incubation mixtures were run on a 5% polyacrylamide gel at 4 °C, and autoradiographed. For competition, the reaction mixture was incubated with ×200 unlabeled AP-1 or Stat3 oligonucleotides before the reaction. For supershift assay, the reaction mixtures were incubated with antic-Jun, anti-JunD or anti-Stat3 before reaction (Santa Cruz Biotechnologies, Santa Cruz, CA).

Statistical analysis

Results are expressed as means ± SD. Significant differences between two groups or more were identified by the unpaired Student *t*-test or Fisher's test, respectively. P-values less than 0.05 were considered statistically significant.

Results

Successful gene transfer and protein expression of Ref-1 in liver grafts using an ex vivo adenoviral gene transfer method

Western blot analysis showed that *ex vivo* transfer of the Ref-1 gene resulted in a significant increase in Ref-1 protein levels in liver tissue 48 h after PLTx. AdLacZ treatment per se resulted in a slight increase of Ref-1 protein compared with PS-treated liver, although a small amount of Ref-1 protein was constitutively expressed in untreated liver as well (Figure 1B).

Recovery of liver graft weight after pLTx

Liver graft weights were increased after PLTx in all groups. In Ref-1 rats, they were increased by 65.2% ± 0.8% on day 3, and 76.3% ± 3.2% on day 5, whereas in the PS- and LacZ-rats the increase was 56.7% ± 3.3% and 52.8% ± 4.2% on day 3, and 67.5% ± 1.7% and 60.4% ± 5.5% on

day 5, respectively. Increased graft weight in Ref-1 rats was statistically significant on both day 3 and day 5. There were no significant differences in liver weight recovery between the PS and LacZ groups (Figure 2).

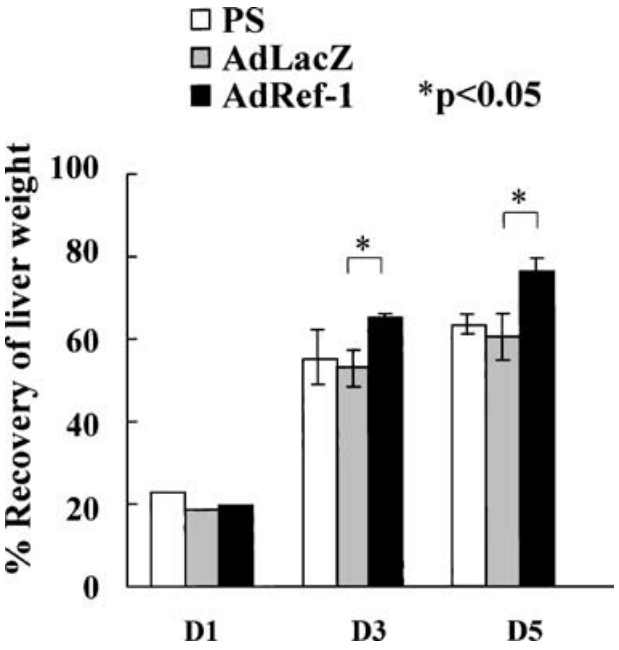

Figure 2: Recovery of liver weight after partial liver transplant (PLTx). Overexpression of redox factor-1 (Ref-1) in 20% liver grafts resulted in a significantly more rapid increase in graft weight on day 3 and day 5 than in the physiological saline (PS) and LacZ groups ($p < 0.05$). The LacZ group showed a slow increase in graft weight compared with the PS-treated group on day 3 and day 5, but there was no significant difference between the two groups. Operations of liver transplantation were performed five times in each group.

BrdU incorporation of hepatocytes

We next performed immunohistochemical analyses using anti-BrdU antibody to investigate the mechanism accounting for Ref-1-mediated increased liver mass. BrdU is a thymidine analog that is incorporated into DNA during the S-phase of the cell cycle, and makes it possible to specifically detect S-phase nuclei. Therefore, it can be used as a marker of the mitogenic activity of hepatocytes. Figure 3 documents a significant increase of BrdU-positive hepatocytes 36 h after transplantation in the Ref-1 group (28.2% ± 4.1%) compared with the PS (17.5% ± 3.4%) and LacZ groups (22.6% ± 1.8%).

Histological study

The histological appearance of the liver after PLTx is shown in Figure 4(A–C). Infiltration of mononuclear cells in periportal areas was observed in the PS- and LacZ-livers, but this was not observed in the Ref-1 liver. The architecture of the liver tissue was conserved as normal, and no apparent necrosis or bleeding was observed in any of the groups.

Proliferation of hepatocytes was estimated by counting mitotic cells 72 h after PLTx. Redox factor-1 introduction resulted in a significant increase in the number of mitotic hepatocytes (F) (1.78% ± 0.19%) compared with the PS (D) (1.1% ± 0.16%) or LacZ groups (E) (1.22% ± 0.15%) (Figures 4A,B).

Improvement of liver injury and function by Ref-1

To examine the effect of overexpressed Ref-1 on liver graft injury induced by I/R, we measured serum levels of ALT on days 3 and 5 after PLTx. Overexpressed Ref-1 significantly reduced the increase of ALT (55 ± 1.2 IU/L) compared with the PS (340 ± 55.6 IU/L) and LacZ (370 ± 96.3 IU/L) groups on day 3 ($p < 0.05$). On day 5, the serum ALT levels returned almost to prehepatectomy levels in the Ref-1

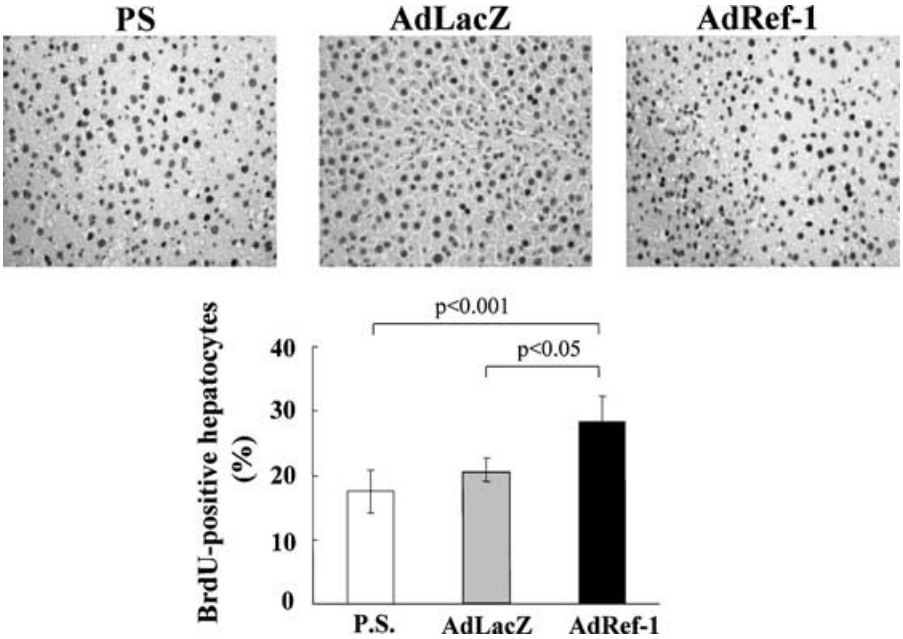

Figure 3: Immunohistochemical analysis of hepatocyte proliferation assessed by bromodeoxyuridine (BrdU) incorporation into the transplanted liver. Overexpressed redox factor-1 (Ref-1) in liver grafts resulted in a significantly increased number of BrdU-positive hepatocyte nuclei compared with the physiological saline (PS) and LacZ groups ($p < 0.05$). Original magnification ×150.

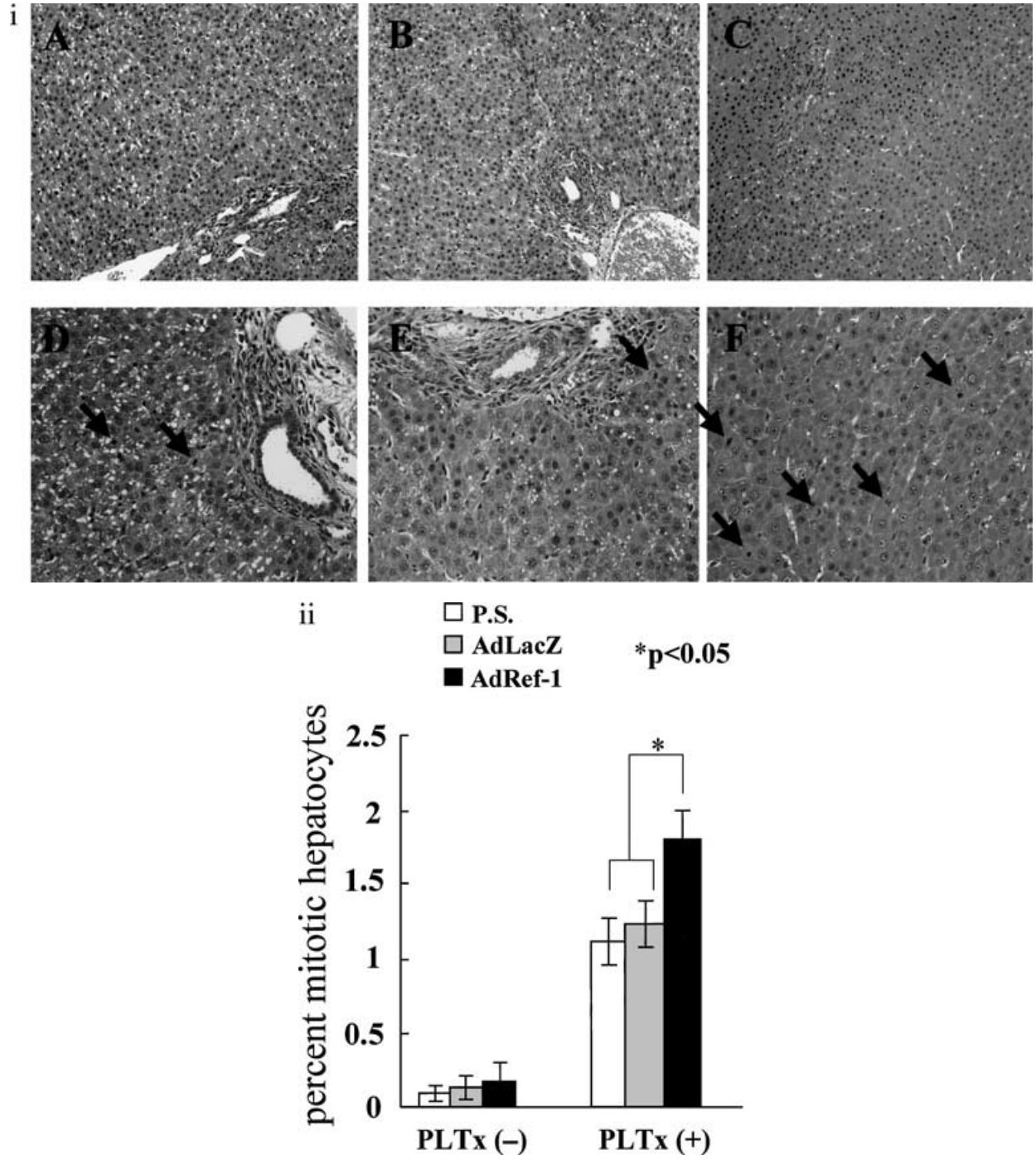

Figure 4: Histological changes in transplanted liver 3 days after partial liver transplant (PLTx). (i) Histological appearance of liver grafts on day 3 after PLTx. Marked perivascular accumulation of mononuclear cells was observed in the physiological saline (PS) (A) and LacZ (B) groups. Slight perivascular infiltration of mononuclear cells was observed in the redox factor-1 (Ref-1) group (C). Normal architecture of the liver tissue was retained and no necrosis or bleeding was observed in any of the groups. Mitotic hepatocytes (→) were observed in the PS (D), LacZ (E) and Ref-1 (F) groups. Original magnification ×150 (A–C), ×300 (D–F). (ii) Percentage of mitotic hepatocytes on day 3 after PLTx. Redox factor-1 introduction resulted in a significant increase in the number of mitotic hepatocytes compared with the PS and LacZ groups ($p < 0.05$).

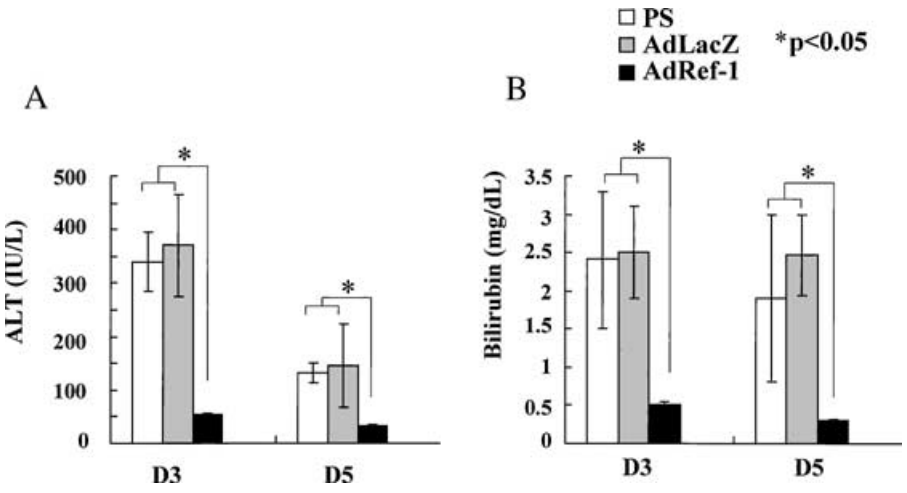

Figure 5: Biochemical analysis of serum alanine aminotransferase (ALT) and total bilirubin levels after partial liver transplant (PLTx). (A) Overexpression of redox factor-1 (Ref-1) was associated with a significantly lower ALT level than in the physiological saline (PS) and LacZ groups on day 3 ($p < 0.05$). On day 5, the serum ALT level returned almost to pre-hepatectomy levels more rapidly in the Ref-1 group than in the PS and LacZ groups ($p < 0.05$). (B) Overexpression of Ref-1 resulted in a significantly lower level of serum bilirubin in the Ref-1 group than in the PS and LacZ groups on days 3 and 5 after PLTx ($p < 0.05$).

group (32 ± 4.2 IU/L), although they were still high in the PS (131 ± 19.4 IU/L) and LacZ (146 ± 78 IU/L) groups ($p < 0.05$) (Figure 5A). Serum levels of bilirubin were used to assess liver function on day 3 and 5 after PLTx, again revealing significantly lower levels of bilirubin in the Ref-1 group (0.5 ± 0.05 mg/dL and 0.3 ± 0.02 mg/dL) than in the PS (2.4 ± 0.9 mg/dL and 1.9 ± 1.1 mg/dL) or LacZ groups (2.5 ± 0.6 mg/dL and 2.47 ± 0.53 mg/dL), respectively ($p < 0.05$) (Figure 5B).

Both liver injury and function as reflected by serum levels of ALT and bilirubin, respectively, may indicate that Ref-1 both protects the liver from injury and preserves its function.

Improved liver regeneration by Ref-1 after partial hepatectomy

We next performed simple 2/3 hepatectomy instead of liver transplantation in order to examine the direct effect of Ref-1 upon liver regeneration, because simple hepatectomy does not accompany ischemia- and/or ischemia/reperfusion-induced injury. Redox factor-1, adenovirally introduced to liver tissue at the time of operation, promoted liver regeneration with significant expression of Ref-1 protein (Figure 6).

These data suggest that Ref-1 possesses the direct effect upon liver regeneration other than the protective effect against I/R-induced injury.

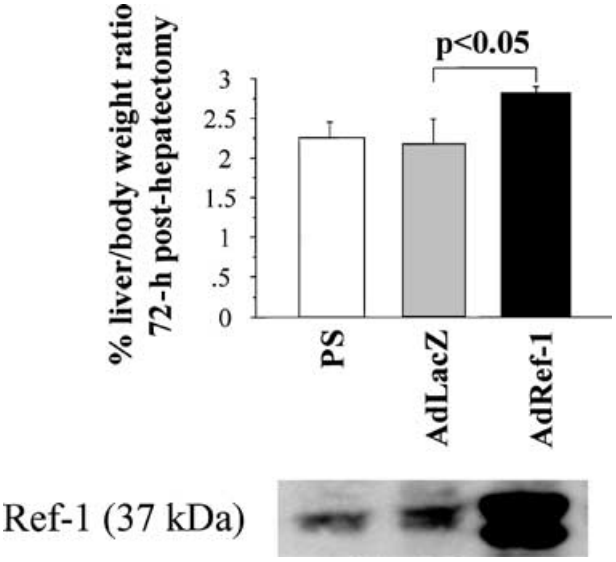

Figure 6: Improvement of liver regeneration by redox factor-1 (Ref-1) after partial hepatectomy in mice. Redox factor-1 adenovirally introduced via tail vein at the time of operation (1×10^9 pfu/100 μL/body) improved liver regeneration after 70% partial hepatectomy. Redox factor-1 protein was sufficiently expressed in mouse liver 72 h after partial hepatectomy in Ref-1 mice. Operations of partial hepatectomy were performed five times in each group. And each immunoblot expresses a representative of three independent experiments.

Ap-1 and Stat3 DNA-binding activities in liver grafts after pLTx

AP-1 and Stat3 DNA-binding activities in the grafts were measured in order to understand the effects of Ref-1 on signal transduction in the liver. Figure 7 (A) shows that AP-1 DNA binding activity was increased transiently on day 1 and recovered to normal levels thereafter in all groups. It was slightly increased only in LacZ livers. Supershift assays revealed that c-Jun and JunD were both contained in the activated AP-1 components. On the other hand, Stat3 DNA-binding in liver tissue was elevated equally from day 1 to day 3 after PLTx (data not shown). Redox factor-1 moderately phosphorylated and activated Stat3 without affecting the amount of Stat3 protein in the tissue (Figure 7C). Stat3 homodimers were also mildly increased in the Ref-1 liver in gelshift assay. Interestingly, Stat1 : Stat3 heterodimers and Stat1 homodimers were also present in PS and LacZ livers, although their signals were comparatively weak. These

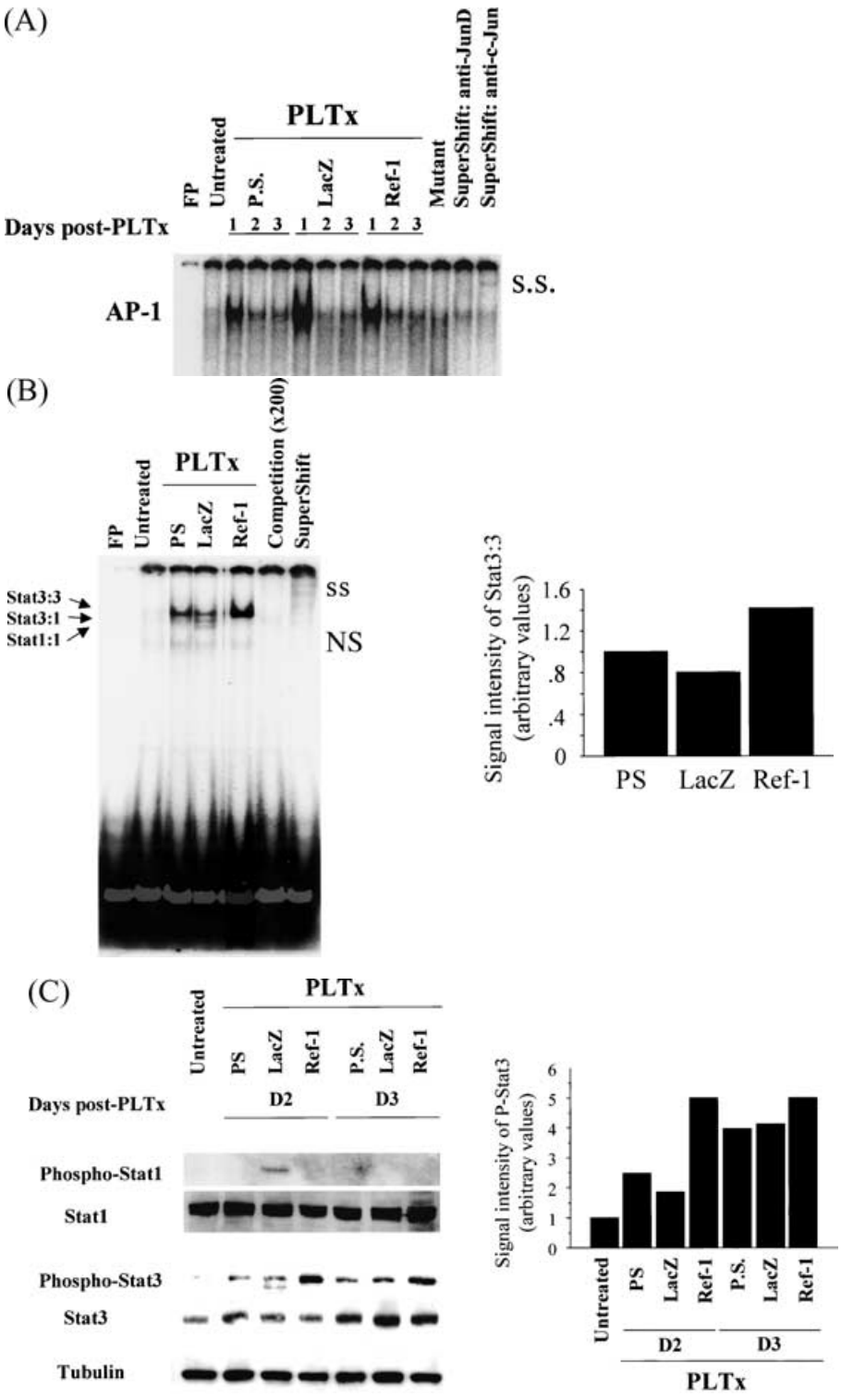

Figure 7: Activator protein-1 (AP-1) and Stat3 DNA-binding activities in liver grafts after partial liver transplant (PLTx). (i) AP-1 DNA binding was examined 1–3 days after liver transplantation in physiological saline (PS), LacZ and redox factor-1 (Ref-1)-treated liver. DNA binding increased on day 1 and decreased thereafter in all groups. Assays using mutant of AP-1 oligonucleotide and supershift by antic-Jun and -JunD were performed for confirming AP-1 signal. No significant difference in AP-1 DNA-binding was observed in any group. FP: free probe, s.s. supershifted band. (ii) Stat3 DNA binding activity 2 days after liver transplantation is shown. Overexpression of Ref-1 resulted in increased Stat3 DNA binding compared with the PS and LacZ groups on day 2 after PLTx. Assays of competition and supershift were performed to confirm Stat signals. (iii) Western blot analysis of Stat1/Stat3 proteins and their phosphorylation in liver grafts on day 2 after PLTx. Each assay expresses a representative of at least three independent experiments.

Stat1-associated signals, however, were not observed in Ref-1-overexpressing liver (Figure 7B). In support of this, Stat1 phosphorylated on tyrosine was clearly observed 2 days after transplantation in the liver of LacZ mice, which was disappeared in Ref-1 liver.

Discussion

Because transfection of target genes using an adenoviral vector administered via the portal vein during liver preservation would be the best choice in terms of both donor's

and recipient's safety, we examined the effectiveness of this approach in a rat model. We have shown that the Ref-1 gene could be introduced into the liver graft where it was functionally active in terms of protein expression. To our knowledge, this is the first report of the efficacy of Ref-1, overexpressed in liver grafts by means of *ex vivo* adenoviral gene transfer, in promoting liver regeneration as well as protecting against postischemic liver injury.

The *ex vivo* gene transfer method during preservation seems to be very effective to obtain sufficient protein expression after transplantation. Additionally, this method may be useful in delivering genes mainly to the liver graft, but not to recipient's other organs. In the Western blot analysis of Ref-1 protein expression after adenoviral gene transfer, two distinct bands were detected (Figure 1B, and Figure 6). The nature of the lower band may be a degradation product or splicing variant of Ref-1.

After 20% PLTx, PS- and LacZ-treated rats showed a comparable slow increase in graft weight. By contrast, overexpression of Ref-1 in hepatic tissue resulted in a rapid increase (recovery) of graft weight on day 3 and day 5 after transplantation, with complete recovery of liver weight 3 days earlier than in the PS and LacZ groups. This rapid increase in liver weight was paralleled by enhanced hepatocyte proliferation, as documented by an increased number of BrdU-positive hepatocytes and mitotic hepatocytes. The significantly increased number of mitotic hepatocytes found in Ref-1 liver grafts implies that the more rapid recovery of graft weight is associated with accelerated cell proliferation. Also in a model of simple hepatectomy where liver damage will not occur, the direct effect of Ref-1 upon liver regeneration was confirmed.

Because Stat3 and AP-1 are important for the homeostatic response during organ repair and are strongly induced during liver regeneration (1,28), and are therefore involved in early response in liver regeneration, we investigated the effects of Ref-1 on their DNA-binding activities. AP-1 DNA-binding activity was increased on day 1 after PLTx and decreased to the baseline level on days 2 and 3 in all three groups. This finding could be interpreted to suggest that increased AP-1 DNA-binding activity on day 1 may not in fact be associated with overexpression of Ref-1, because peak production of the Ref-1 protein occurred on day 3 after transfection (although Ref-1 was already weakly expressed 24 h after transplantation, data not shown). Redox factor-1 may thus have little effect on AP-1 activation in the post-transplant liver in this experiment model, although activated AP-1 components include c-Jun/JunD, which may be involved in cell proliferation after PLTx.

Enhanced Stat3 DNA-binding on day 2 in Ref-1 liver suggests that Ref-1 activates Stat3 DNA-binding and that this is responsible for the significant increase in graft weight and hepatocyte proliferation, although the mechanism for this remains unclear. Interestingly, overexpression of Ref-1 enhanced Stat3 homodimer formation, but reduced Stat1-associated homo- or hetero-dimer formation (Stat1 : 1 or Stat1 : 3). Stat1-associated signals observed in ischemic and/or postischemic organs may be related to induction of apoptotic cell death, as reported previously (29–31). Redox factor-1 induction may inhibit apoptotic cell death by suppressing these Stat1-related signals, which would contribute to the prevention of liver injury and the increase of liver mass. We have previously reported that overexpression of Ref-1 in the liver inhibits postischemic oxidative cell death and tissue injury *in vivo* (11). The results of the present study are consistent with our previous findings. However, it remains to be elucidated whether the central effect of Ref-1 on the transplanted liver is prevention of postischemic injury and/or promotion of regeneration.

In conclusion, the results of the present study provide the first evidence that Ref-1 participates in the regulation of liver graft regeneration. Redox factor-1 introduced into liver grafts by adenoviral vectors not only protected them from ischemia/reperfusion-induced injury, but accelerated regeneration in a partial liver transplant model. Although the precise mechanisms of these effects of Ref-1 on liver grafts require further clarification, this study may provide a new approach to resolve the problem of small-size liver graft transplantation.

Acknowledgments

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