

UCLA

UCLA Previously Published Works

Title

Heme oxygenase-1 regulates sirtuin-1-autophagy pathway in liver transplantation: From mouse to human

Permalink

<https://escholarship.org/uc/item/30m505zn>

Journal

American Journal of Transplantation, 18(5)

ISSN

1600-6135

Authors

Nakamura, Kojiro
Kageyama, Shoichi
Yue, Shi
[et al.](#)

Publication Date

2018-05-01


DOI

10.1111/ajt.14586

Peer reviewed

ORIGINAL ARTICLE

Heme oxygenase-1 regulates sirtuin-1–autophagy pathway in liver transplantation: From mouse to human

Kojiro Nakamura¹ | Shoichi Kageyama¹ | Shi Yue¹ | Jing Huang¹ |
 Takehiro Fujii¹ | Bibo Ke¹ | Rebecca A. Sosa²  | Elaine F. Reed² | Nakul Datta¹ |
 Ali Zarrinpar¹ | Ronald W. Busuttil¹ | Jerzy W. Kupiec-Weglinski¹

¹The Dumont-UCLA Transplant Center, Department of Surgery, Division of Liver and Pancreas Transplantation, University of California, Los Angeles, Los Angeles, CA, USA

²Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA

Correspondence

Jerzy W. Kupiec-Weglinski
 Email: jkupiec@mednet.ucla.edu

Present addresses

Shi Yue, Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research at USC, Department of Stem Cell Biology and Regenerative Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

Jing Huang, Department of Hepatobiliary-Pancreatic Surgery, Lihuli Hospital, Ningbo University School of Medicine, Ningbo, China

Ali Zarrinpar, Department of Surgery, Division of Transplantation and Hepatobiliary Surgery, University of Florida College of Medicine, Gainesville, FL, USA

Funding information

Center for Scientific Review, Grant/Award Number: PO1 AI120944, R01 DK102110, R01 DK107533, R01 DK06

Liver ischemia–reperfusion injury (IRI) represents a major risk factor of early graft dysfunction and a key obstacle to expanding the donor pool in orthotopic liver transplantation (OLT). Although graft autophagy is essential for resistance against hepatic IRI, its significance in clinical OLT remains unknown. Despite recent data identifying heme oxygenase-1 (HO-1) as a putative autophagy inducer, its role in OLT and interactions with sirtuin-1 (SIRT1), a key autophagy regulator, have not been studied. We aimed to examine HO-1–mediated autophagy induction in human OLT and in a murine OLT model with extended (20 hours) cold storage, as well as to analyze the requirement for SIRT1 in autophagy regulation by HO-1. Fifty-one hepatic biopsy specimens from OLT patients were collected under an institutional review board protocol 2 hours after portal reperfusion, followed by Western blot analyses. High HO-1 levels correlated with well-preserved hepatocellular function and enhanced SIRT1/LC3B expression. In mice, HO-1 overexpression by genetically modified HO-1 macrophage therapy was accompanied by decreased OLT damage and increased SIRT1/LC3B expression, whereas adjunctive inhibition of SIRT1 signaling diminished HO-1–mediated hepatoprotection and autophagy induction. Our translational study confirms the clinical relevance of HO-1 cytoprotection and identifies SIRT1-mediated autophagy pathway as a new essential regulator of HO-1 function in IR-stressed OLT.

KEYWORDS

basic (laboratory) research/science, biopsy, immunobiology, liver disease: immune/inflammatory, liver transplantation/hepatology, organ perfusion and preservation, tissue injury and repair, translational research/science

1 | INTRODUCTION

Orthotopic liver transplantation (OLT) is the standard of care for patients with end-stage liver disease and those with hepatic

malignancies, but liver graft shortage remains a major challenge. Liver ischemia–reperfusion injury (IRI), an innate immune-driven sterile inflammation response leading to hepatocellular death, is an inevitable consequence of multiple clinical conditions, including trauma, sepsis,

Abbreviations: Ad, adenovirus; ALT, alanine aminotransferase; BMI, body mass index; BMM, bone marrow–derived macrophages; Bx, biopsy; COPP, cobalt protoporphyrin; EAD, early allograft dysfunction; HE, hematoxylin and eosin; HO-1, heme oxygenase-1; IL, interleukin; IRI, ischemia–reperfusion injury; IR, ischemia–reperfusion; OLT, orthotopic liver transplantation; POD, postoperative day; sALT, serum alanine aminotransferase; SIRT1, sirtuin 1; TGF, transforming growth factor; TUNEL, TdT-mediated dUTP nick end labeling; UCLA, University of California, Los Angeles; UW, University of Wisconsin.

Kojiro Nakamura, Shoichi Kageyama, and Shi Yue contributed equally.

hepatic tumor resection, and liver transplantation. Indeed, hepatic IRI has been recognized as a major risk factor for delayed early graft function and acute and chronic rejection as well as a key obstacle to expanding the donor organ pool. However, despite obvious clinical importance, mechanisms that account for liver IRI are only partially understood and no effective therapy is available to prevent or treat this condition in humans.¹

Autophagy is an evolutionarily conserved intracellular self-digesting pathway responsible for maintaining energy homeostasis and removing long-lived or damaged organelles and proteins.² A growing body of evidence indicates a tissue-protective function for autophagy in various pathologic states, such as aging, diabetes, and neurodegenerative diseases,³ as well as in liver IRI.⁴⁻⁶ Although enhanced autophagy may represent a novel therapeutic target against IR damage, its efficacy and mechanism in OLT settings remain to be assessed, while clinical relevance of autophagy pathway in liver transplant patients has not been studied before.

Heme oxygenase-1 (HO-1; *Hmox1*), a rate-limiting enzyme that catalyzes the conversion of heme into biliverdin, carbon monoxide, and free iron, exerts potent antioxidative, anti-inflammatory, and cytoprotective functions.⁷ We and others have reported on the beneficial function of HO-1 in hepatic IRI murine models by using chemical HO-1 inducers,⁸⁻¹¹ adenovirus (Ad) gene transfer,^{8,12} and genetically modified animals.¹³ Because macrophages are key mediators of innate immune-driven inflammation and the primary source of HO-1 in ischemia-reperfusion (IR)-stressed liver,^{10,14} we have established a novel molecular transfer approach by using ex vivo genetically modified HO-1-overexpressing macrophages. This regimen effectively transferred the target molecule into 40-50% of hepatic cells and successfully alleviated warm IRI in mice^{15,16}; its putative efficacy in clinically relevant OLT models has not been tested. Although anti-inflammatory phenotype is central to HO-1 function in liver IRI,¹⁷ recent studies have identified HO-1 as a novel hepatic autophagy inducer^{18,19}; its role in liver transplantation remains unknown, and putative regulatory mechanisms need to be studied.

Sirtuin 1 (SIRT1), an NAD⁺-dependent type III histone/protein deacetylase involved in cellular senescence, inflammation, and stress resistance,²⁰ plays a key role in autophagy induction.^{21,22} In addition to an anti-inflammatory role in the pathogenesis of liver IRI,²³ recent studies have demonstrated the significance of SIRT1-induced autophagy in hepatocyte resistance against IR stress.^{24,25} Molecular communication between SIRT1 and HO-1 in OLT has not been studied, and the mechanism by which SIRT1 may regulate the HO-1 autophagy pathway remains to be elucidated.

To gain further insight into HO-1-mediated autophagy in liver transplantation, we used an ex vivo genetically modified HO-1 macrophage adoptive transfer approach in a clinically relevant murine OLT model and analyzed human OLT samples (N = 51) in parallel. Our translational study confirms the clinical relevance of HO-1 hepatoprotection and identifies SIRT1-dependent autophagy as a novel and essential regulator of HO-1 function in OLT under IR stress.

2 | MATERIALS AND METHODS

2.1 | Clinical liver transplant study

Fifty-six consecutive adult primary OLT recipients were recruited under an institutional review board protocol (13-000143; May 2013-August 2015). Routine standard of care and immunosuppressive therapy were administered as specified by University of California, Los Angeles (UCLA) liver transplant protocols. Study data were collected and managed by using REDCap electronic data capture tools.²⁶ Donor organs, procured according to standardized techniques, were perfused with and stored in cold University of Wisconsin (UW) solution (ViaSpan; Bristol-Myers Squibb Pharma, New York, NY). Cold ischemia time was defined as the time between the perfusion of the donor liver with UW solution and its removal from cold storage. Recipient venous blood was collected within the hour before transplantation and on postoperative days (PODs) 1-14. Hepatocellular injury was evaluated based on serum alanine aminotransferase (sALT) levels. Posttransplantation biopsy (Bx) specimens were obtained from the left liver lobe by using a Tru-Cut biopsy needle (Carefusion, Medline Industries, Northfield, IL) approximately 2 hours after portal reperfusion (before abdominal closure) and then snap-frozen. Of 56 cases, 51 were examined; 4 were excluded because the Bx samples were too small for Western blot analyses, and 1 was excluded due to unavailability of clinical data. Early allograft dysfunction (EAD) was defined by the presence of ≥ 1 of the following: total bilirubin ≥ 10 mg/dL (171 μ mol/L) on POD 7, international normalized ratio ≥ 1.6 on POD 7, and alanine aminotransferase/aspartate aminotransferase >2000 IU/L within the first 7 PODs.^{27,28} Posttransplantation rejection was diagnosed by follow-up Bx performed per clinical standard of care.

2.2 | Animals

C57BL/6 mice aged 6-8 weeks were used (Jackson Laboratory, Bar Harbor, ME). Animals were housed in the UCLA animal facility under specific pathogen-free conditions and received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health publication 86-23 revised 1985). All studies were reviewed and approved by the UCLA Animal Research Committee.

2.3 | Generation of genetically modified bone marrow-derived macrophages

L929 cells (ATCC, Rockville, MD) were cultured in RPMI-1640 medium supplemented with 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 μ g/mL streptomycin, and 10% fetal calf serum. The conditioned medium was collected from cells grown for 7 days. The bone marrow-derived macrophages (BMMs) were generated according to standard procedures.¹⁵ In brief, bone marrow cells were removed from the femurs and tibias of C57BL/6 mice and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 U/mL penicillin, 100 μ g/mL streptomycin, and 2 mmol/L L-glutamine, with

the addition of 15% L929-conditioned medium. Cells were cultured at 5×10^6 cells/well for 7 days with 5% CO₂-95% air at 37°C and used for in vitro transfection.

Replication-defective recombinant Ad encoding HO-1 (AdHO-1) was generated as described.²⁹ BMMs (5×10^6 /well) were incubated for 1 hour with AdHO-1 or Ad β -gal (at a multiplicity of infection of 10). After medium change, cells were cultured for additional 48 hours before in vivo adoptive transfer. By 48 hours of transfection, the X-Gal staining was >90%, compared with control BMMs.¹⁵

2.4 | Mouse liver cold ischemia and transplantation model

We used a well-established mouse model of ex vivo hepatic cold storage and orthotopic liver transplantation (OLT) as described.³⁰ Donor livers were stored in UW solution at 4°C for 20 hours before transplantation into syngeneic mice. BMMs (5×10^6) transfected with AdHO-1/Ad β -gal (2.5×10^9 pfu) or untreated (5×10^6) were injected via portal vein before liver reperfusion at the completion of transplant surgery. In some experiments, SIRT1 inhibitor (EX527; Sigma Aldrich, St. Louis, MO) was administered (10 mg/kg i.p.) at 30 minutes before OLT surgery.^{15,16} Liver and serum samples were collected 6 hours after reperfusion, the peak of hepatocellular damage in this model. The sham group underwent the same procedures except for OLT.

2.5 | Serum biochemistry

sALT levels, an indicator of hepatocellular injury, were measured at IDEXX Laboratories (Westbrook, ME).

2.6 | Liver histology and IRI grading

Formalin-fixed paraffin-embedded OLT sections (5 μ m) were stained with hematoxylin and eosin (HE). The severity of hepatic IRI was graded by using the Suzuki's criteria.³¹

2.7 | Quantitative RT-PCR analysis

RNA was extracted from liver tissue samples using the RNase Mini Kit (Qiagen, Germantown, MD). A total of 5.0 μ g of RNA was reverse-transcribed into cDNA. Quantitative PCR was performed using DNA Engine with Chromo 4 Detector (MJ Research, Waltham, MA).³² The primer sequences are listed (Table S1). The expression of the target gene was normalized to the housekeeping gene *HPRT*.

2.8 | Western blot assay

Proteins were extracted from liver tissues, and their concentrations were measured (BCA Protein Assay Kit, Thermo Scientific). Equal amounts of protein were electrophoresed, blotted, and incubated with primary Abs and secondary HRP-conjugated Abs and developed. Primary Abs detecting HO-1 (Enzo Life Sciences, Farmingdale, NY), SIRT1, LC3B, and β -actin (Cell Signaling Technology, Danvers, MA)

were used. To compare target protein expression in multiple human OLT samples, densitometry quantification was conducted by using a reference sample and normalization with β -actin as reported.²³

2.9 | Immunohistochemistry

OLT-infiltrating neutrophils were detected by using monoclonal rat anti-Ly6G Abs (BD Biosciences, San Jose, CA). Immunostaining signals were visualized with a labeled polymer in the EnVision+ Kit (HRP; Dako, Carpinteria, CA). Positively stained cells were counted blindly (10 HPF/section). LC3B expression in OLT was detected by using rabbit anti-LC3B Abs, and signals were visualized with secondary Abs: Alexa Fluor 488 anti-rabbit IgG.

2.10 | TdT-mediated dUTP nick end labeling (TUNEL) assay

Cell death in formalin-fixed paraffin-embedded liver sections (5 μ m) was detected by use of the ApopTag Plus Peroxidase In Situ Apoptosis Kit (Millipore, Temecula, CA). Results were scored semiquantitatively by blindly counting the number of positive cells in 10 HPF/section.

2.11 | Statistical analysis

In experiments in mice, group comparisons were performed by using a Student *t*-test. For human data, continuous values were analyzed by using a Mann-Whitney *U* test, and categorical variables were analyzed by using the Fisher exact test. The Spearman correlation coefficient (*r*) was used to evaluate the strength of linear relationship between variables. The cumulative survival rate was analyzed by using the Kaplan-Meier method, and differences between groups were compared by using a log-rank test. JMP for Windows 8.0 (SAS Institute, Cary, NC) was used for statistical analyses. A *P*-value of <.05 was considered statistically significant.

3 | RESULTS

3.1 | AdHO-1-transfected BMMs alleviate IRI in OLT

We first aimed to validate the efficacy of HO-1 induction after the adoptive transfer of AdHO-1 BMMs in a clinically relevant mouse IRI-OLT model. Cold-stored liver grafts were infused with BMMs via portal vein immediately before reperfusion. At 6 hours after reperfusion, AdHO-1-transfected BMM-treated OLT showed increased HO-1 expression compared with naive or Ad β -gal-transfected BMM-treated controls (Figure 1A). AdHO-1 BMM-conditioned OLT displayed attenuated sinusoidal congestion, edema/vacuolization, and hepatocellular necrosis compared with control OLT infused with untreated or Ad β -gal-transfected cells (Figure 1B). These correlated with depressed Suzuki's grading of histologic liver damage (OLT: + AdHO-1 BMMs = 1.7 ± 0.6 vs OLT: + BMMs = 3.7 ± 1.2 or OLT: +Ad β -gal BMMs = 3.3 ± 0.6 ; *n* = 4-6/group, *P* < .05, Figure 1C) and

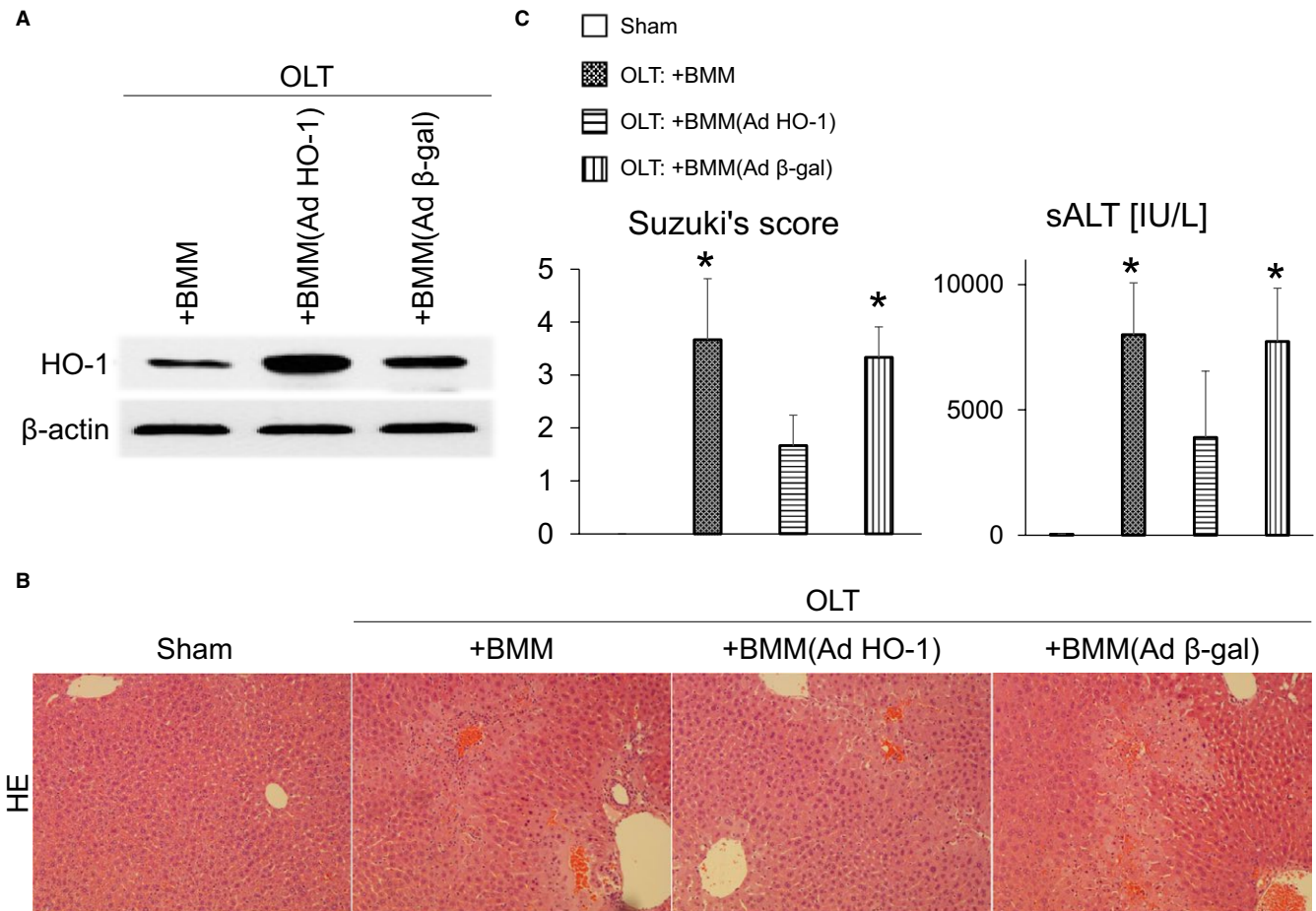


FIGURE 1 Adoptive transfer of adenovirus (Ad) heme oxygenase-1 (HO-1) bone marrow-derived macrophages (BMMs) ameliorates ischemia-reperfusion (IR) damage in orthotopic liver transplantation (OLT). Mouse (C57BL/6) livers subjected to 20 hours of cold storage were transplanted into syngeneic mice. BMMs (5×10^6) transfected with AdHO-1 or Ad β -gal (2.5×10^9 pfu) or untreated BMMs were infused into liver grafts via portal vein immediately before reperfusion. Liver grafts and serum samples were analyzed at 6 hours post-OLT. A, Representative ($n = 3$) Western blot-assisted detection of HO-1; β -actin expression served as an internal control. B, Representative H&E staining ($n = 3$; original magnification, $\times 100$). C, Suzuki's histologic grading of liver IRI and sALT levels ($n = 4-6$ /group; $*P < .05$ vs OLT: + BMM [AdHO-1]). Data shown are mean \pm SD

preserved liver function, evidenced by sALT levels (OLT: + AdHO-1 BMMs = 3900 ± 2642 vs. OLT: + BMMs = 7997 ± 2068 or OLT: +Ad β -gal BMMs = 7726 ± 2129 IU/L; mean \pm SD, $n = 4-6$ /group, $P < .05$, Figure 1C) in AdHO-1 BMM-treated OLT. Thus, infusion of AdHO-1 BMMs into the graft enhanced HO-1 expression and minimized the adverse effects of hepatic IRI in OLT.

3.2 | AdHO-1 gene transfer promotes anti-inflammatory signature in OLT

Because anti-inflammatory function is one of the key HO-1 biological features,¹⁷ we examined whether AdHO-1 gene transfer alters the anti-inflammatory/proinflammatory gene expression profile in IR-stressed OLT. Indeed, AdHO-1 BMMs infused directly into the graft increased mRNA coding for anti-inflammatory interleukin (IL)-10 and transforming growth factor (TGF) β , while decreasing proinflammatory TNF α , MCP1, and CXCL10 compared with naïve/Ad β -gal BMM-treated controls (Figure 2A,B). This confirms that HO-1 overexpression exerts

an anti-inflammatory function and inhibits inflammatory cytokine-induced OLT injury. We also tested whether AdHO-1 gene transfer may affect cytokine-induced neutrophil OLT influx. As expected, HO-1 overexpression reduced neutrophil (Ly6G) numbers in IR-stressed OLT infused with AdHO-1 BMMs (Figure 3A,B), indicating HO-1 gene transfer enhanced the anti-inflammatory phenotype and diminished cytokine-induced inflammation.

3.3 | AdHO-1 gene transfer attenuates hepatocellular death while enhancing SIRT1/LC3B expression in OLT

We next aimed to determine whether AdHO-1 gene transfer affects cell death and autophagic activity in IR-stressed OLT. As shown in Figure 3A,B, a reduced frequency of TUNEL-positive apoptotic/necrotic cell death was observed in AdHO-1 BMM-treated OLT compared with naïve BMM or Ad β -gal BMM controls. Moreover, we found that AdHO-1 BMM therapy not only upregulated hepatic SIRT1

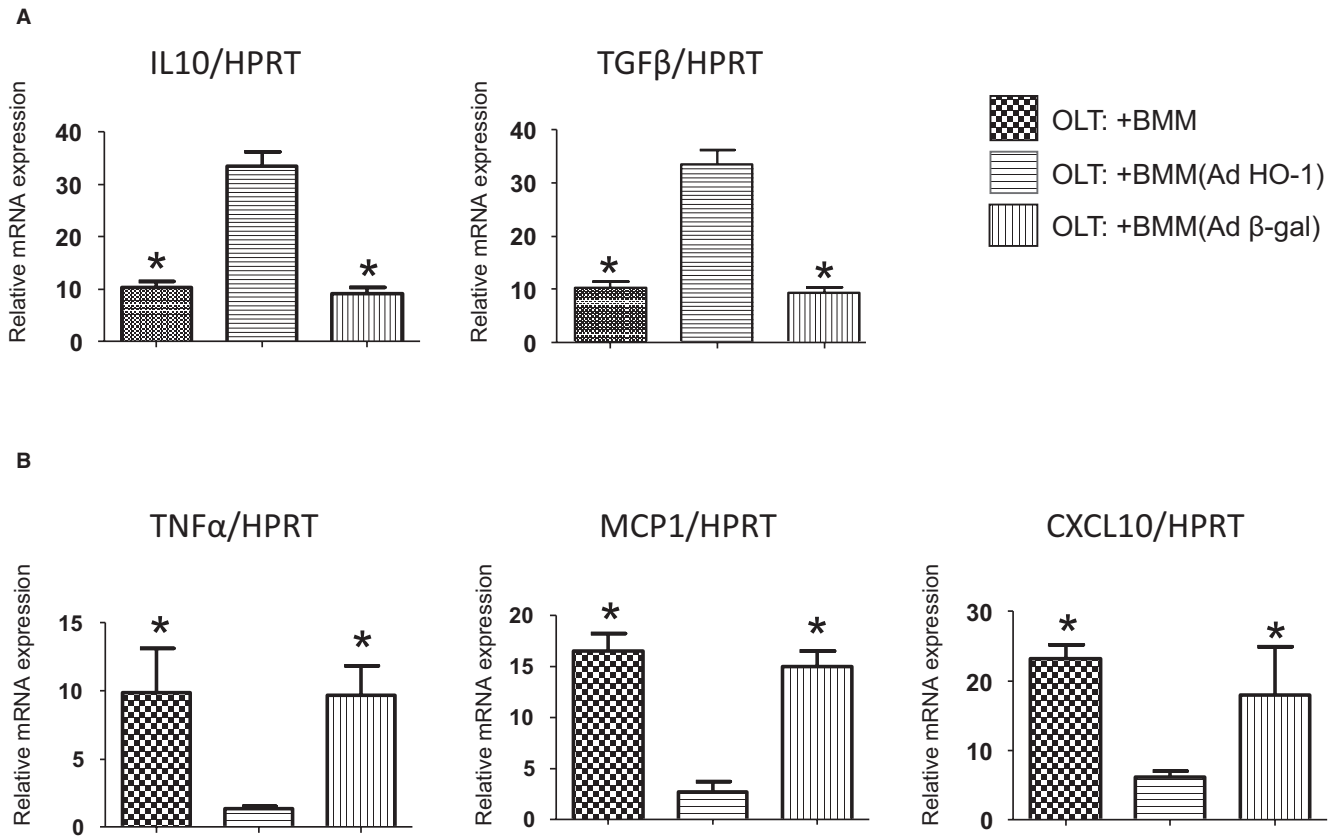


FIGURE 2 Adoptive transfer of adenovirus (Ad) heme oxygenase-1 (HO-1) bone marrow–derived macrophages (BMMs) inhibits proinflammatory signature and enhances anti-inflammatory program in orthotopic liver transplantation (OLT). Mouse (C57BL/6) livers subjected to 20 hours of cold storage and transplanted into syngeneic mice were infused immediately before reperfusion via portal vein with BMMs (naive, AdHO-1, or Ad β-gal). Samples were analyzed at 6 hours post-OLT. qRT-PCR–assisted detection of mRNA coding for interleukin (IL)-10 and transforming growth factor (TGF)β (A) and TNFα, MCP1, and CXCL10 (B). Data were normalized to *HPRT* gene expression ($n = 4/\text{group}$; * $P < .05$ vs OLT: + BMM [AdHO-1]). Data shown are mean \pm SD

(a key autophagy regulator) but also enhanced LC3B (an autophagosome marker) expression, compared with controls (Figure 4A-C), underscoring the importance of the HO-1–SIRT1 axis in cell autophagy regulation. Taken together, these results are consistent with the idea that HO-1 overexpression exerts cytoprotective and autophagic functions in IR-stressed OLT.

3.4 | HO-1 expression correlates with IR liver damage and SIRT1/LC3B signaling in human OLT

Having demonstrated AdHO-1 BMM-facilitated hepatoprotection accompanied by increased SIRT1/LC3B levels in a murine OLT model, we next aimed to validate the relevance of these findings in human liver transplantation. Liver postreperfusion Bx specimens from 51 human OLTs were screened by Western blots for HO-1, SIRT1, and LC3B expression (Figure 5A). Hepatic HO-1 expression profile exhibited weak negative correlation with sALT levels at POD1 ($r = -0.3306$, $P = .0178$, Figure 5B), indicating higher levels of HO-1 were associated with milder IR-liver injury. In addition, graft HO-1 expression showed positive correlation with SIRT1 ($r = 0.4229$, $P = .0020$, Figure 5C) and weak positive correlation with LC3B ($r = 0.3594$, $P = .0096$, Figure 5D).

To evaluate the impact of hepatic HO-1 on clinical outcomes, 51 OLT samples were split evenly into low ($n = 26$) and high ($n = 25$) HO-1 expression groups (Figure 6A). The patients' demographic data and clinical parameters are shown (Table S2). There was no correlation between HO-1 expression and donor background, including age, gender, weight, body mass index (BMI), or preprocurement sALT level. We also found no correlation between HO-1 expression and recipient factors, including age, gender, weight, BMI, race, disease etiology, presence of hepatocellular carcinoma, ABO-compatibility, Model for End-Stage Liver Disease score, pretransplantation sALT, or dialysis. Furthermore, there was no correlation between HO-1 expression and cold ischemic time or intraoperative blood loss.

Consistent with Figure 5C,D data, the high-HO-1 group showed increased SIRT1 (low HO-1: 0.83 ± 0.10 vs high HO-1: 1.25 ± 0.10 , mean \pm SEM, $P < .05$, Figure 6B, left) and LC3B (low HO-1: 0.87 ± 0.08 vs high HO-1: 1.13 ± 0.10 , mean \pm SEM, $P < .05$, Figure 6B, right) expression. Representative Western blots are shown (Figure 6C). In addition, the high-HO-1 group exhibited significantly lower sALT levels at POD1 (low HO-1: 809 ± 367 vs high HO-1: 255 ± 34 IU/L, mean \pm SEM, $P < .05$, Figure 6D). To examine the relationship between HO-1 expression and OLT outcomes, we analyzed cumulative posttransplantation survival,

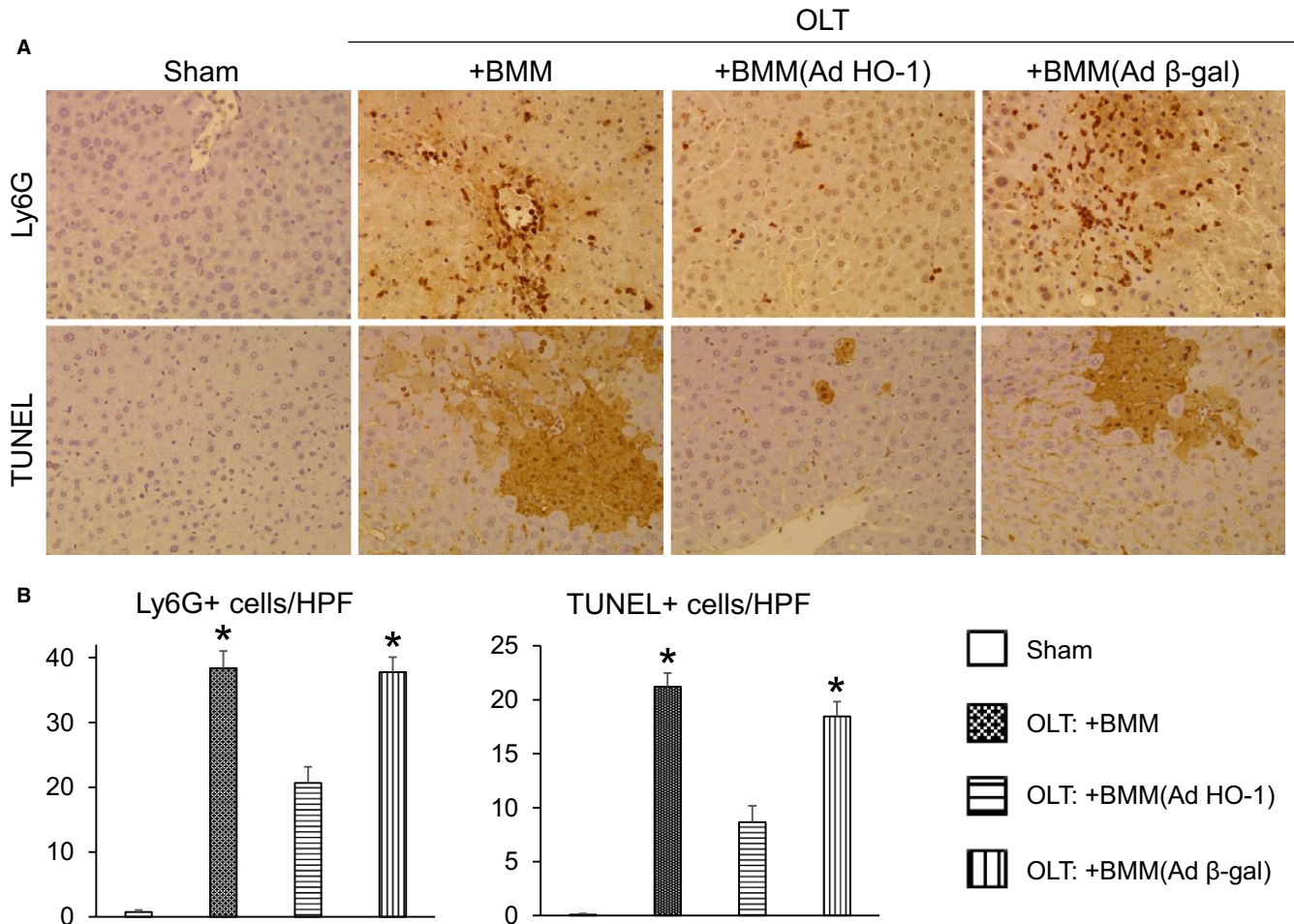


FIGURE 3 Adoptive transfer of adenovirus (Ad)heme oxygenase-1 (HO-1) bone marrow-derived macrophages (BMMs) suppresses neutrophil trafficking and mitigates hepatocellular death in orthotopic liver transplantation (OLT). Mouse (C57BL/6) livers subjected to 20 hours of cold storage and transplanted into syngeneic mice were infused via portal vein with BMMs (naive, AdHO-1, or Ad β -gal). Samples were analyzed at 6 hours post-OLT. A, Representative immunohistochemical Ly6G (upper panels) and TdT-mediated dUTP nick end labeling (TUNEL) (lower panels) staining. B, Quantification of Ly6G- (left) and TUNEL- (right) positive cells/HPF ($n = 4-6$ /group; $*P < .05$ vs OLT: + BMM [AdHO-1]). Data shown are mean \pm SD

with the median follow-up of 740 days (range, 4-1432 days). None of the patients underwent secondary liver transplantation. Despite consistent trends, the improved survival in the high-HO-1 expression group failed to reach statistical significance compared with the low-HO-1 expression group ($P = .1963$, Figure 6E). The high-HO-1 expression group was characterized by lower frequency of early graft dysfunction (3.8% vs 8.0%) and post-OLT rejection episodes (3.8% vs 16.0%). However, these differences failed to reach statistical significance ($P = .5150$ and $P = .1871$, respectively). Representative TUNEL and LC3B staining in low- versus high-HO-1 Bx groups are shown (Figure 6F).

3.5 | SIRT1 inhibition abrogates hepatoprotection and depresses autophagy despite HO-1 induction in mouse OLT

Based on the aforementioned preclinical and clinical data, preservation of hepatocellular function (sALT) and enhanced autophagy (LC3B) in HO-1-overexpressing OLT was accompanied by increased SIRT1

signaling, a putative regulator of autophagy and stress resistance against hepatic IR damage.²⁵ Hence, we hypothesized SIRT1 might be essential in cytoprotection after HO-1 induction in IR-stressed OLT. As shown in Figure 7A, adjunctive SIRT1 inhibition before transplantation (EX527, 10 mg/kg i.p.) recreated cardinal features of hepatic IRI in otherwise well-functioning AdHO-1 BMM-treated OLT, evidenced by increased sALT levels and Suzuki's histologic grading scores (Figure 7B). Moreover, SIRT1 inhibition depressed LC3B expression in AdHO-1 BMM-treated liver grafts. These data indicate the SIRT1-autophagy pathway is required for HO-1-mediated hepatoprotection against IRI in OLT.

4 | DISCUSSION

This study is the first to document HO-1-mediated autophagy enhancement in human liver transplant settings and in a clinically relevant mouse OLT model. Although anti-inflammatory signaling is

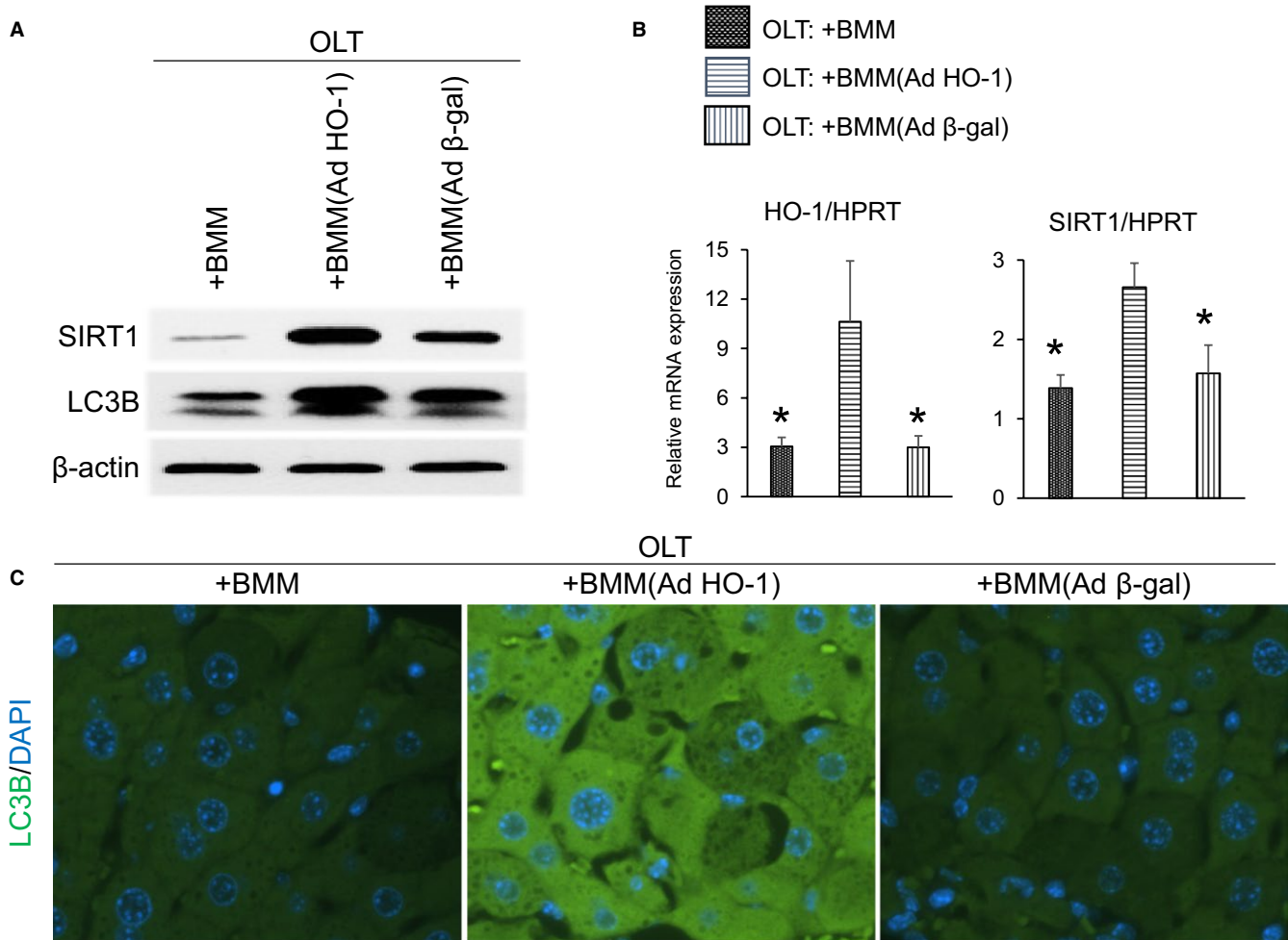


FIGURE 4 Adoptive transfer of adenovirus (Ad)heme oxygenase-1 (HO-1) bone marrow-derived macrophages (BMMs) enhances SIRT1/LC3B expression in orthotopic liver transplantation (OLT). Mouse (C57BL/6) livers subjected to 20 hours of cold storage and transplanted to syngeneic mice were infused via portal vein with BMMs (naive, AdHO-1, or Ad β -gal). Samples were analyzed at 6 hours post-OLT. A, Representative (n = 3) Western blot-assisted detection of SIRT1 and LC3B; β -actin expression served as an internal control. B, qRT-PCR-assisted detection of mRNA coding for HO-1 and SIRT1. Data were normalized to *HPRT* gene expression (n = 4/group; * P < .05 vs OLT: + BMM (AdHO-1). Data shown as mean \pm SD. C, Representative (n = 3) immunohistochemical detection of LC3B in OLT at 6 hours post-reperfusion

critical for HO-1 function against IR stress, our findings document autophagy regulation as a novel mechanistic component of its biological effects. Our clinical study showed positive correlation between HO-1 and SIRT1 levels in human OLT (Figures 5C,D and 6B,C), while SIRT1 inhibition in parallel mouse studies diminished autophagy phenotype seen otherwise after HO-1 induction in IR-resistant OLT (Figures 4A,B 7C,D). These data are consistent with the notion that HO-1 regulates hepatic autophagy in an SIRT1-dependent manner. Although previous studies suggested the role of p38 MAPK in HO-1-induced liver autophagy,^{18,33} it remains unknown as to how HO-1 may regulate hepatic p38 signaling. In the context of our present data, a recent finding of liver-specific SIRT1 increasing p38 translocation/phosphorylation in a YAP/MKK3-dependent manner³⁴ implies SIRT1-p38 signaling may underlie the autophagy regulation by HO-1. These complex molecular mechanisms require further in-depth analyses.

It is well documented that hepatocyte autophagy is essential in liver stress resistance.^{4,35,36} Indeed, the loss of Atg4B increased

sensitivity of aged mouse livers to warm IRI,⁵ whereas rapamycin increased autophagy and protected against IR stress.⁶ In contrast, recent studies in myeloid-specific Atg5 knockout mice have shed new light on macrophage autophagy regulation.³⁷⁻³⁹ While macrophage Atg5 deficiency was shown to limit acute toxic liver injury by downregulating IL-1 β ,³⁸ the impaired macrophage Atg5 promoted proinflammatory macrophage polarization in obese mice.³⁷ Although macrophages are pivotal in innate immune-driven IR damage, the significance of macrophage autophagy in liver IRI, using myeloid autophagy-deficient animals, awaits future research.

Despite numerous preclinical reports identifying HO-1 as a major cytoprotective molecule,^{10,15,16} Geuken and coworkers reported high "pre-transplant" HO-1 expression in human donor livers paradoxically correlated with augmented posttransplantation injury.⁴⁰ As "post-transplant" HO-1 features specific to clinical liver transplantation have not been well defined, we have recently reported that high HO-1 expression in human OLT (n = 21) at the time of reperfusion are

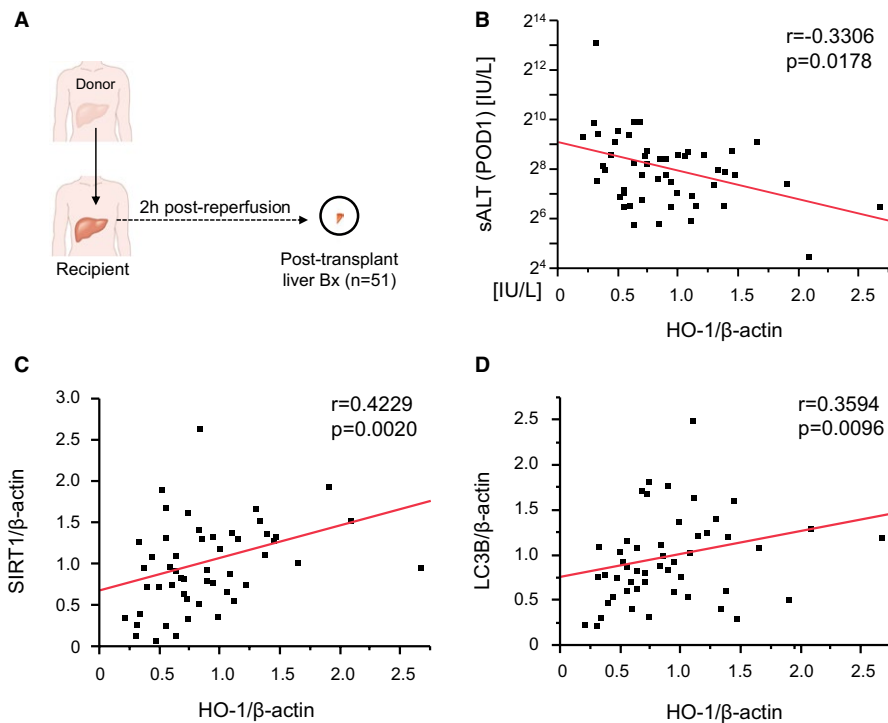


FIGURE 5 Posttransplantation heme oxygenase-1 (HO-1) expression negatively correlates with hepatocellular damage and positively with SIRT1/LC3B expression in human orthotopic liver transplantation (OLT). A, Posttransplantation liver biopsies (Bx; 2 hours postreperfusion) were collected from 51 OLT patients. Bx samples were analyzed by Western blotting with β -actin normalization. B, Relationship between posttransplantation HO-1 expression and serum alanine aminotransferase (sALT) levels at postoperative day 1 (POD1). C, D, Relationship between posttransplantation HO-1 and SIRT1 (C) and LC3B (D) expression. r is the Spearman correlation coefficient

associated with suppressed IR liver damage.⁴¹ In agreement with the latter, our present data from human OLT recipients ($n = 51$) consistently demonstrated negative correlation between HO-1 levels and posttransplantation sALT levels (Figures 5B and 6D), implying therapeutic benefit of HO-1 induction in clinical liver transplantation. Unlike hepatocytes under basal conditions, liver macrophages (infiltrating and tissue resident) are the major HO-1 producers, in both human OLT and IR-stressed murine livers.^{10,41}

Although aging and obesity are poor prognostic factors influencing HO-1 expression, we found no correlation between posttransplantation HO-1 levels and donor–recipient demographic parameters or surgical factors (Table S2). Because HO-1 is not only a cytoprotective molecule but also stress-inducible heat shock protein 32 (hsp32), its expression increases with the severity of IR stress. Paradoxically, high HO-1 levels were associated with more attenuated liver damage in the clinical arm of our study (Figure 6D). We speculate that the divergent HO-1 expression in OLT might be caused by varying individual inductivity of HO-1 in response to IR stress, resulting in varying susceptibility against IRI. Indeed, short guanine-thymine nucleotide repeats polymorphism in the promotor region on chromosome 22q13.1 of human HO-1 was shown to enhance HO-1 expression in response to oxidative stress,⁴² whereas a more recent study associated HO-1 polymorphism with post-OLT hepatocellular damage.⁴³ As others failed to correlate HO-1 polymorphism with its expression profile,⁴⁰ more studies on putative factors influencing human HO-1 responses are warranted.

We have also recently reported on the anti-inflammatory and hepatoprotective role of SIRT1 activation in a warm liver IRI mouse model alongside negative correlation between SIRT1 and T-bet/IL-1 β levels in 21 clinical OLT cases.²³ The positive SIRT1 regulation by HO-1 seen in a mouse IRI model was then confirmed in a 21-OLT patient

cohort.⁴¹ The current study documents the functional significance of the HO-1–SIRT1 axis in a clinically relevant mouse hepatic cold ischemia/OLT model (Figure 4A,B) and in 51 human OLT cases (Figures 5C and 6B). Although in our previous clinical study ($n = 21$), post-OLT “high” HO-1 expression was accompanied by a statistically significant improvement in OLT survival,⁴¹ we failed to detect a similar statistically significant survival difference in the current ($n = 51$) liver transplant patient cohort (Figure 6E, $P = .1963$). As discussed earlier,⁴¹ the link between graft and recipient long-term outcomes and IRI severity remains controversial. The presence of early lesions on the time-zero Bx in older grafts ($n = 16$) did not translate into early graft dysfunction or graft loss,⁴⁴ while histologic IRI grading in postreperfusion liver Bxs and peak transaminase levels ($n = 55$) did not show any correlation with clinical outcomes in the first posttransplantation month.⁴⁵ Likewise, despite preferable trends, our present study ($n = 51$) failed to detect significant differences in EAD, post-OLT rejection, or overall survival. In marked contrast, severe IRI on the time-zero Bx and peak of posttransplantation ALT in 476 OLT cases were significantly associated with a 1-year graft survival in an earlier study.⁴⁶ The correlation between graft/recipient outcomes and IRI severity may thus require considerably larger number of study subjects.

A chemical HO-1 inducer (cobalt protoporphyrin [COPP]) was recently shown to increase SIRT1 expression in hepatocytes, while adjunctive competitive HO-1 inhibitor (tin mesoporphyrin) abrogated SIRT1 upregulation, indicating HO-1–dependent SIRT1 induction by exogenous COPP.^{47,48} Likewise, in our current study, transfer of genetically modified HO-1–overexpressing BMMs consistently enhanced liver graft SIRT1 expression levels (Figure 4A,B). The mechanisms accounting for HO-1–mediated SIRT1 upregulation remain largely unknown. Because oxidative stress suppresses hepatic SIRT1,^{23,49}

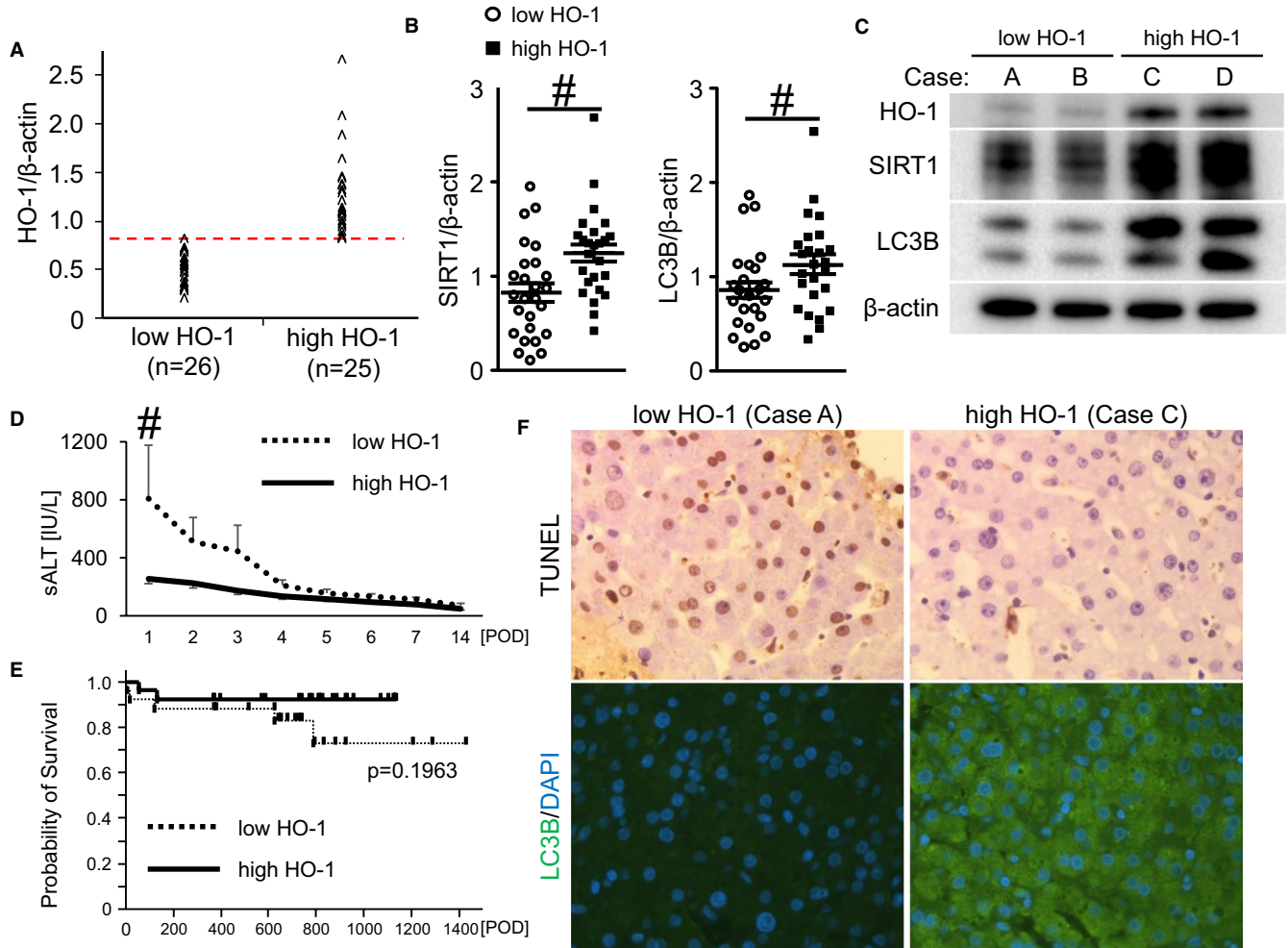


FIGURE 6 Increased posttransplantation heme oxygenase-1 (HO-1) levels correlate with attenuated hepatocellular damage and enhanced SIRT1-LC3B pathway in human orthotopic liver transplantation (OLT). A, Biopsy samples collected from the 51 OLT patients were classified into low-HO-1 (n = 26) and high-HO-1 (n = 25) groups. B, Western blot-assisted expression of SIRT1 and LC3B. Data shown in dot plots and bars indicate mean \pm SEM. $^{\#}P < .05$ (Mann-Whitney *U* test). C, Representative Western blot-assisted detection of HO-1, SIRT1, and LC3B (case A/B: low-HO-1 group; case C/D: high-HO-1 group). D, Serum alanine aminotransferase (sALT) levels at postoperative days 1-14 (PODs1-14) shown as mean \pm SEM. Dotted line indicates low HO-1, while the solid line indicates high HO-1. $^{\#}P < .05$ (Mann-Whitney *U* test). E, The cumulative probability of OLT survival (Kaplan-Meier method). Dotted line indicates low HO-1, while the solid line indicates high HO-1 groups (log-rank test). F, Representative TdT-mediated dUTP nick end labeling (TUNEL) and LC3B staining pattern in OLT

antioxidant properties may be the key for HO-1 to regulate SIRT1 expression.

Ad is an efficient vector for *in vivo* gene therapy due to its ability to mediate transgene expression in various cell types. However, serious off-target effects, including activation of innate immune responses, thrombocytopenia, coagulopathy, and liver damage, restrict systemic use of Ad vector in clinical practice.⁵⁰ In our present and previous preclinical studies,^{15,16} we used replication-defective recombinant Ad to generate HO-1-overexpressing BMMs. Indeed, the *ex vivo* gene-modified local BMM infusion upregulated hepatic HO-1/LC3B expression and alleviated IRI in a mouse OLT model. This regimen makes Ad-related adverse effects negligible,⁵¹ while direct BMM infusion into the liver improves macrophage transfer efficacy as a cellular delivery system for HO-1 trafficking into the disease site.⁵² The present study using *ex vivo* gene-modified HO-1-overexpressing BMM in a

clinically relevant mouse model is likely to validate and support future experiments in translational and clinical settings.

In our current study, the injection of BMMs (Ad β -gal) to OLT increased hepatic HO-1 expression compared with untreated BMM (no virus)-treated graft (Figure 1A). This result is consistent with our previous findings in a mouse model of liver warm IRI.¹⁶ As a foreign pathogen, virus infection itself can cause cellular stress to activate antioxidant system, accompanied by increased antioxidant gene expression, such as manganese superoxide dismutase, HO-1, indoleamine-2,3-dioxygenase, and glutathione peroxidase.⁵³ We speculate that increased HO-1 expression following Ad β -gal BMM injection was induced by virus-related BMM stress, which was accompanied by increased SIRT1 and slightly higher LC3B levels (Figure 4A). However, Ad β -gal BMMs failed to ameliorate liver damage in IR-stressed OLT (Figure 1B,C) as "pure" viral-mediated HO-1 induction may not be

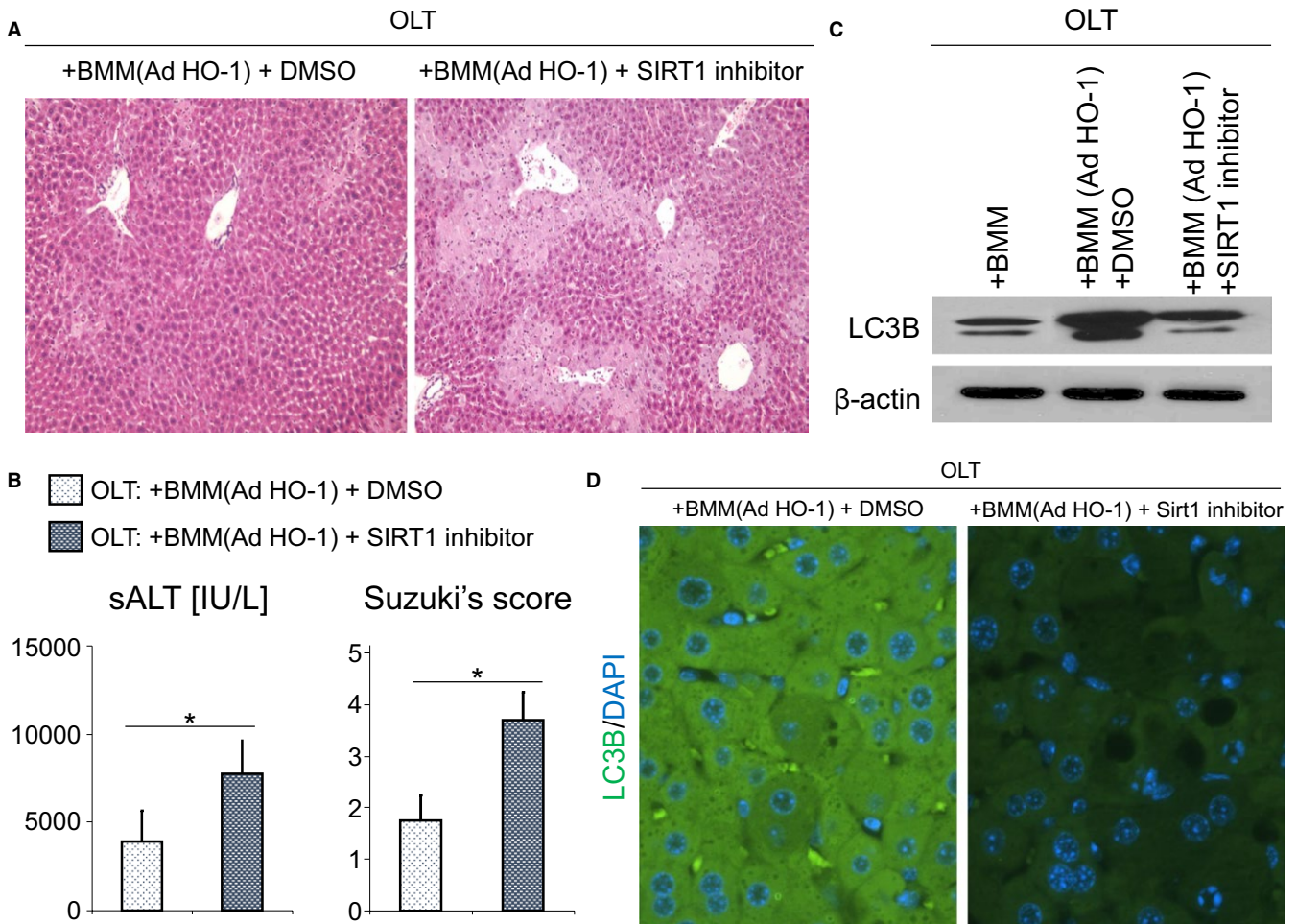


FIGURE 7 SIRT1 inhibition depresses LC3B expression and abrogates hepatoprotection in adenovirus (Ad)heme oxygenase-1 (HO-1) bone marrow-derived macrophage (BMM)-conditioned ischemia-reperfusion (IR) orthotopic liver transplantation (OLT). Mouse (C57BL/6) livers subjected to 20 hours of cold storage were transplanted into syngeneic mice and conditioned with AdHO-1 BMMs with or without adjunctive SIRT1 inhibition (EX527, 10 mg/kg i.p). Samples were analyzed at 6 hours post-OLT. **A**, Representative H&E staining (original magnification, $\times 100$). **B**, Serum alanine aminotransferase (sALT) levels (left) and Suzuki's histologic grading of liver IRI (right) ($n = 3-4$ /group; $*P < .05$ vs OLT: + AdHO-1 BMM + DMSO). Data shown are mean \pm SD. **C**, Representative ($n = 3$) Western blot-assisted detection of LC3B; β -actin expression served as an internal control. **D**, Representative ($n = 3$) immunohistochemical detection of LC3B

sufficiently proficient to protect the liver graft. In contrast, unlike the Ad β -gal BMM controls, infusion of Ad HO-1 BMMs markedly enhanced local HO-1 (Figure 1A) and SIRT1/LC3B (Figure 4A) levels, leading to attenuated hepatocellular injury in IR-stressed OLT (Figure 1B,C).

In conclusion, our study with genetically modified HO-1-overexpressing BMM transfer in a mouse OLT model and parallel clinical examination of 51 human liver transplants not only confirmed the clinical relevance of HO-1 hepatoprotection but also identified a novel regulatory role of SIRT1-mediated autophagy as a part of cytoprotective function in transplant recipients. The present data, in agreement with our recent reports,^{15,16} validate a novel investigative tool in which host macrophages can be transfected *ex vivo* with cytoprotective HO-1 and then infused, if needed, into prospective recipients to mitigate IR-mediated inflammation during liver transplantation, resection, or trauma. As decreasing donor organ quality represents one of the most challenging problems, our findings provide the rationale for a new and clinically attractive gene-targeted strategy to "rejuvenate"

extended criteria donor livers and improve their function via an HO-1-SIRT1-autophagy pathway in transplant recipients.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health (NIH) grants R01 DK102110; R01 DK107533; and R01 DK062357 (JWKW); NIH PO1 A1120944 and Keck Foundation Award 986722 (JWKW and EFR); and Ruth L. Kirschstein National Research Service Award T32CA009120 (RAS). The authors declare that no conflicts of interest exist. We thank Ko Takashi at UCLA-TPCL for immunohistochemical assistance.

DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

ORCID

Rebecca A. Sosa  <http://orcid.org/0000-0002-9860-6830>

REFERENCES

- Zhai Y, Petrowsky H, Hong JC, Busuttil RW, Kupiec-Weglinski JW. Ischaemia-reperfusion injury in liver transplantation – from bench to bedside. *Nat Rev Gastroenterol Hepatol*. 2013;10(2):79-89.
- Baehrecke EH. Autophagy: dual roles in life and death? *Nat Rev Mol Cell Biol*. 2005;6(6):505-510.
- Czaja MJ, Ding WX, Donohue TM Jr, et al. Functions of autophagy in normal and diseased liver. *Autophagy*. 2013;9(8):1131-1158.
- Kim JS, Nitta T, Mohuczy D, et al. Impaired autophagy: a mechanism of mitochondrial dysfunction in anoxic rat hepatocytes. *Hepatology*. 2008;47(5):1725-1736.
- Wang JH, Ahn IS, Fischer TD, et al. Autophagy suppresses age-dependent ischemia and reperfusion injury in livers of mice. *Gastroenterology*. 2011;141(6):2188-2199.e2186
- Zhu J, Lu T, Yue S, et al. Rapamycin protection of livers from ischemia and reperfusion injury is dependent on both autophagy induction and mammalian target of rapamycin complex 2-Akt activation. *Transplantation*. 2015;99(1):48-55.
- Otterbein LE, Soares MP, Yamashita K, Bach FH. Heme oxygenase-1: unleashing the protective properties of heme. *Trends Immunol*. 2003;24(8):449-455.
- Amersi F, Buelow R, Kato H, et al. Upregulation of heme oxygenase-1 protects genetically fat Zucker rat livers from ischemia/reperfusion injury. *J Clin Invest*. 1999;104(11):1631-1639.
- Tamura T, Kondo T, Ogawa K, Fukunaga K, Ohkohchi N. Protective effect of heme oxygenase-1 on hepatic ischemia-reperfusion injury through inhibition of platelet adhesion to the sinusoids. *J Gastroenterol Hepatol*. 2013;28(4):700-706.
- Kato H, Amersi F, Buelow R, et al. Heme oxygenase-1 overexpression protects rat livers from ischemia/reperfusion injury with extended cold preservation. *Am J Transplant*. 2001;1(2):121-128.
- Tsuchihashi S, Zhai Y, Bo Q, Busuttil RW, Kupiec-Weglinski JW. Heme oxygenase-1 mediated cytoprotection against liver ischemia and reperfusion injury: inhibition of type-1 interferon signaling. *Transplantation*. 2007;83(12):1628-1634.
- Ke B, Buelow R, Shen XD, et al. Heme oxygenase 1 gene transfer prevents CD95/Fas ligand-mediated apoptosis and improves liver allograft survival via carbon monoxide signaling pathway. *Hum Gene Ther*. 2002;13(10):1189-1199.
- Tsuchihashi S, Livhits M, Zhai Y, Busuttil RW, Araujo JA, Kupiec-Weglinski JW. Basal rather than induced heme oxygenase-1 levels are crucial in the antioxidant cytoprotection. *J Immunol*. 2006;177(7):4749-4757.
- Patel A, van de Poll MC, Greve JW, et al. Early stress protein gene expression in a human model of ischemic preconditioning. *Transplantation*. 2004;78(10):1479-1487.
- Ke B, Shen XD, Gao F, et al. Adoptive transfer of ex vivo HO-1 modified bone marrow-derived macrophages prevents liver ischemia and reperfusion injury. *Mol Ther*. 2010;18(5):1019-1025.
- Huang J, Shen XD, Yue S, et al. Adoptive transfer of heme oxygenase-1 (HO-1)-modified macrophages rescues the nuclear factor erythroid 2-related factor (Nrf2) antiinflammatory phenotype in liver ischemia/reperfusion injury. *Mol Med*. 2014;20:448-455.
- Ke B, Shen XD, Ji H, et al. HO-1-STAT3 axis in mouse liver ischemia/reperfusion injury: regulation of TLR4 innate responses through PI3K/PTEN signaling. *J Hepatol*. 2012;56(2):359-366.
- Carchman EH, Rao J, Loughran PA, Rosengart MR, Zuckerbraun BS. Heme oxygenase-1-mediated autophagy protects against hepatocyte cell death and hepatic injury from infection/sepsis in mice. *Hepatology*. 2011;53(6):2053-2062.
- Liu A, Huang L, Guo E, et al. Baicalein pretreatment reduces liver ischemia/reperfusion injury via induction of autophagy in rats. *Sci Rep*. 2016;6:25042.
- Finkel T, Deng CX, Mostoslavsky R. Recent progress in the biology and physiology of sirtuins. *Nature*. 2009;460(7255):587-591.
- Ou X, Lee MR, Huang X, Messina-Graham S, Broxmeyer HE. SIRT1 positively regulates autophagy and mitochondria function in embryonic stem cells under oxidative stress. *Stem Cells*. 2014;32(5):1183-1194.
- Sathyanarayan A, Mashek MT, Mashek DG. ATGL promotes autophagy/lipophagy via SIRT1 to control hepatic lipid droplet catabolism. *Cell Rep*. 2017;19(1):1-9.
- Nakamura K, Kageyama S, Ke B, et al. Sirtuin 1 attenuates inflammation and hepatocellular damage in liver transplant ischemia-reperfusion: from mouse-to-human. *Liver Transpl*. 2017;23:1282-1293.
- Rickenbacher A, Jang JH, Limani P, et al. Fasting protects liver from ischemic injury through Sirt1-mediated downregulation of circulating HMGB1 in mice. *J Hepatol*. 2014;61(2):301-308.
- Biel TG, Lee S, Flores-Toro JA, et al. Sirtuin 1 suppresses mitochondrial dysfunction of ischemic mouse livers in a mitofusin 2-dependent manner. *Cell Death Differ*. 2016;23(2):279-290.
- Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381.
- Deschenes M, Belle SH, Krom RA, Zetterman RK, Lake JR. Early allograft dysfunction after liver transplantation: a definition and predictors of outcome. National Institute of Diabetes and Digestive and Kidney Diseases Liver Transplantation Database. *Transplantation*. 1998;66(3):302-310.
- Olthoff KM, Kulik L, Samstein B, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transpl*. 2010;16(8):943-949.
- Shibahara S, Muller R, Taguchi H, Yoshida T. Cloning and expression of cDNA for rat heme oxygenase. *Proc Natl Acad Sci USA*. 1985;82(23):7865-7869.
- Shen XD, Gao F, Ke B, et al. Inflammatory responses in a new mouse model of prolonged hepatic cold ischemia followed by arterialized orthotopic liver transplantation. *Liver Transpl*. 2005;11(10):1273-1281.
- Suzuki S, Toledo-Pereyra LH, Rodriguez FJ, Cejalvo D. Neutrophil infiltration as an important factor in liver ischemia and reperfusion injury. Modulating effects of FK506 and cyclosporine. *Transplantation*. 1993;55(6):1265-1272.
- Kamo N, Ke B, Ghaffari AA, et al. ASC/caspase-1/IL-1beta signaling triggers inflammatory responses by promoting HMGB1 induction in liver ischemia/reperfusion injury. *Hepatology*. 2013;58(1):351-362.
- Wang Y, Shen J, Xiong X, et al. Remote ischemic preconditioning protects against liver ischemia-reperfusion injury via heme oxygenase-1-induced autophagy. *PLoS ONE*. 2014;9(6):e98834.
- Wang Y, Cui R, Zhang X, et al. SIRT1 increases YAP- and MKK3-dependent p38 phosphorylation in mouse liver and human hepatocellular carcinoma. *Oncotarget*. 2016;7(10):11284-11298.
- Ding WX, Li M, Chen X, et al. Autophagy reduces acute ethanol-induced hepatotoxicity and steatosis in mice. *Gastroenterology*. 2010;139(5):1740-1752.
- Ni HM, Bockus A, Boggess N, Jaeschke H, Ding WX. Activation of autophagy protects against acetaminophen-induced hepatotoxicity. *Hepatology*. 2012;55(1):222-232.
- Liu K, Zhao E, Ilyas G, et al. Impaired macrophage autophagy increases the immune response in obese mice by promoting proinflammatory macrophage polarization. *Autophagy*. 2015;11(2):271-284.
- Ilyas G, Zhao E, Liu K, et al. Macrophage autophagy limits acute toxic liver injury in mice through down regulation of interleukin-1beta. *J Hepatol*. 2016;64(1):118-127.

39. Lodder J, Denaes T, Chobert MN, et al. Macrophage autophagy protects against liver fibrosis in mice. *Autophagy*. 2015;11(8):1280-1292.
40. Geuken E, Buis CI, Visser DS, et al. Expression of heme oxygenase-1 in human livers before transplantation correlates with graft injury and function after transplantation. *Am J Transplant*. 2005;5(8):1875-1885.
41. Nakamura K, Zhang M, Kageyama S, et al. Macrophage HO-1-SIRT1-p53 Axis Regulates Sterile Inflammation in Liver Ischemia-Reperfusion Injury. *J Hepatol*. 2017;67(6):1232-1242.
42. Hirai H, Kubo H, Yamaya M, et al. Microsatellite polymorphism in heme oxygenase-1 gene promoter is associated with susceptibility to oxidant-induced apoptosis in lymphoblastoid cell lines. *Blood*. 2003;102(5):1619-1621.
43. Zhang ZY, Guan J, Li H, Zhou ZQ, Zhou GW. Heme oxygenase-1 promoter polymorphism protects liver allograft. *Indian J Surg*. 2016;78(1):14-19.
44. Deschenes M, Forbes C, Tchervenkov J, et al. Use of older donor livers is associated with more extensive ischemic damage on intraoperative biopsies during liver transplantation. *Liver Transpl Surg*. 1999;5(5):357-361.
45. Berberat PO, Friess H, Schmied B, et al. Differentially expressed genes in postperfusion biopsies predict early graft dysfunction after liver transplantation. *Transplantation*. 2006;82(5):699-704.
46. Ali JM, Davies SE, Brais RJ, et al. Analysis of ischemia/reperfusion injury in time-zero biopsies predicts liver allograft outcomes. *Liver Transpl*. 2015;21(4):487-499.
47. Liu X, Gao Y, Li M, et al. Sirt1 mediates the effect of the heme oxygenase inducer, cobalt protoporphyrin, on ameliorating liver metabolic damage caused by a high-fat diet. *J Hepatol*. 2015;63(3):713-721.
48. Sodhi K, Puri N, Favero G, et al. Fructose mediated non-alcoholic fatty liver is attenuated by HO-1-SIRT1 module in murine hepatocytes and mice fed a high fructose diet. *PLoS ONE*. 2015;10(6):e0128648.
49. Chung S, Yao H, Caito S, Hwang JW, Arunachalam G, Rahman I. Regulation of SIRT1 in cellular functions: role of polyphenols. *Arch Biochem Biophys*. 2010;501(1):79-90.
50. Appledorn DM, McBride A, Seregin S, et al. Complex interactions with several arms of the complement system dictate innate and humoral immunity to adenoviral vectors. *Gene Ther*. 2008;15(24):1606-1617.
51. Froh M, Wheeler MD, Smutney O, Zhong Z, Bradford BU, Thurman RG. New method of delivering gene-altered Kupffer cells to rat liver: studies in an ischemia-reperfusion model. *Gastroenterology*. 2003;124(1):172-183.
52. Burke B, Sumner S, Maitland N, Lewis CE. Macrophages in gene therapy: cellular delivery vehicles and in vivo targets. *J Leukoc Biol*. 2002;72(3):417-428.
53. Choi AM, Knobil K, Otterbein SL, Eastman DA, Jacoby DB. Oxidant stress responses in influenza virus pneumonia: gene expression and transcription factor activation. *Am J Physiol*. 1996;271(3 Pt 1):L383-L391.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Nakamura K, Kageyama S, Yue S, et al. Heme oxygenase-1 regulates sirtuin-1-autophagy pathway in liver transplantation: From mouse to human. *Am J Transplant*. 2017;00:1-12. <https://doi.org/10.1111/ajt.14586>