Supplemental contents

S1. Flow cytometry

Flow cytometry was performed to characterize the phenotypes of ADSCs. ADSCs at passage 3 isolated from F344/NCLs rats were labeled with fluorescein isothiocyanate-conjugated anti-rat CD29 antibody (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), anti-rat CD31 antibody (Thermo Fisher Scientific Inc., Waltham, MA, USA), anti-rat CD44 antibody (Thermo Fisher Scientific Inc.), anti-rat CD45 antibody (Thermo Fisher Scientific Inc.), anti-rat CD73 antibody (Bioss, Inc., Woburn, MA, USA), and anti-rat CD90 antibody (Becton, Dickinson and Company) for 60 min on ice. Labeled cells were measured by a BD AccuriTM C6 Flow Cytometer (BD Biosciences, Franklin Lakes, NJ, USA) and the obtained data were analyzed by BD AccuriTM C6 (BD Biosciences).

ADSCs isolated from adipose tissues of F344/NSLs rats adhered to the plastic dishes and had a typical spindle shape, similar to fibroblasts. Flow cytometry demonstrated that CD29 and CD44 were positively expressed, CD90 was moderately expressed, CD73 was slightly expressed, while CD31 and CD45
(hematopoietic and endothelial markers) were negatively expressed in rat ADSCs (see below).
**S2. Immunohistochemistry**

In the renal subcapsular transplantation model, hematoxylin and eosin (HE) staining and cytokeratin 18 staining, using anti-cytokeratin 18 antibodies (ab181597, Abcam plc, Cambridge, UK) with goat anti-rabbit IgG HRP (K4003, Agilent Technologies Inc. Santa Clara, CA, USA) as secondary antibody, was performed to detect transplanted hepatocytes. To evaluate the proliferation promoting effect of ADSCs on hepatocyte grafts, 5-bromo-2'-deoxyuridine (BrdU) staining was performed. BrdU (Abcam plc) (100 mg/kg) was intraperitoneally injected in the HTx (\(n = 4\)) and CoTx (\(n = 4\)) groups at 17, 18, 19, 24, 25, and 26 days after transplantation, and kidneys were retrieved on day 28. BrdU and cytokeratin 18 double staining was performed using anti-BrdU antibody-HRP (Abcam plc) and anti-Cytokeratin 18 antibodies (Abcam plc) with SK5100 Vector Red (Vector Laboratories Inc., Burlingame, CA, USA). The number of cytokeratin 18 and BrdU-double positive cells and cytokeratin 18-positive cells were counted. The BrdU-positive rate (cytokeratin 18 and BrdU-double positive cells/cytokeratin 18-positive cells) was calculated. In the CoTx group, cytokeratin 18-positive cells were divided into 2 subgroups (contact (+) with kidney parenchyma (KP (+) group, \(n = 4\)), contact (-) with kidney parenchyma (KP (-) group, \(n = 4\)), and the BrdU-
positive rate in each group was calculated. The count was performed by a
pathologist using a blind method. In the portal vein transplantation model, HE
staining and albumin staining was performed using anti-albumin antibodies (MP
Biomedicals, Santa Ana, CA, USA) combined with the VECTASTAIN ABC
system (Vector Laboratories, Inc.).
S3. Identification of key factors for the hepatocyte function in the ADSC co-culture supernatant in an in vitro model

ADSC co-culture with hepatocytes was performed to investigate the effect on the hepatocyte function, and culture supernatants were analyzed to identify the key factors of the above effect. Cells were seeded on a type 1 collagen-coated 6-well plate (CORNING, Kennebunk, ME, USA) or insert with 1.0 μm pores (CORNING) at a density of 5.0×10⁵ cells/well. Hepatocytes alone (Hepatocyte group, \( n = 21 \)), ADSCs alone (ADSC group, \( n = 21 \)), hepatocytes together with ADSCs (Co-culture group, \( n = 21 \)), and hepatocytes indirectly together with ADSCs using an insert (Ind Co-culture group, \( n = 21 \)) were cultured for 3 days. Inserts were placed in all groups and 4 ml of Williams medium E (gibco, Thermo Fisher Scientific Inc.) containing 1% Pe-St was used as the medium. An ammonia removal assay was performed to evaluate the hepatocyte function, as previously reported. The IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17, IL-18, IFN-γ, TNF-α, Eotaxin, MCP-1, IP-10, GRO/KC, GM-CSF, VEGF, EGF, Fractalkine, LIX, MIP-1α, MIP-2 and RANTES levels in culture supernatant (\( n = 14 \), respectively) were measured with a Milliplex MAP Rat Cytokine/chemokine Magnetic Bead Panel (Merck KGaA, Darmstadt, Germany) using a Bioplex 200
system (Bio-Rad, Hercules, CA, USA). HGF levels were quantified using a Mouse/Rat HGF Quantikine ELISA Kit (R&D Systems, Minneapolis, Minnesota, USA).
**S4. Inhibition of candidate factors in co-culture supernatant**

Co-culture supernatants were used to perform the inhibition assay for candidate key factors. After 3 days of co-culture, supernatants were collected and centrifuged at 1000G for 3 min and filtered with a 0.2 μm filter (Sartorius, Gottingen, Germany) to remove the bacteria. Co-culture supernatants were divided into 2 groups mainly based on lot-to-lot variations: (1) effective for increasing the hepatocyte function (Co-cul sup (E) group); and (2) not effective for increasing the hepatocyte function (Co-cul sup (IE) group). At first, hepatocytes (1.0×10^5) were seeded on a type I collagen-coated 24-well plate (CORNING) in 0.8 ml of the following medium: basal medium (WE group, n = 14), hepatocyte supernatant (Hep sup group, n = 14), ADSC supernatant (ADSC sup group, n = 14), effective co-culture supernatant (Co-cul sup (E) group, n = 8), or ineffective co-culture supernatant (Co-cul sup (IE) group, n = 6). Then, in the inhibition assay for the key factors, only the co-culture supernatants in the Co-cul sup (E) group were used. Hepatocytes (1.0×10^5) were seeded on a 24-well plate in 0.8 ml of Co-cul sup (E) supernatant, including anti-HGF antibodies (anti-HGF group, n = 8) (1.0 μg/ml) (R&D Systems), anti-VEGF antibodies (anti-VEGF group, n = 8) (1.0 μg/ml) (R&D Systems), anti-IL-6 antibodies (anti-IL-6 group, n = 8)
(3.0 μg/ml) (R&D Systems), or IgG isotype control (IgG group, \( n = 8 \)) (1.0 μg/ml) (R&D Systems). Before hepatocyte seeding, neutralizing antibodies were added to the co-culture supernatants, and incubated for 30 min at 37°C under 5% CO₂. After 3 days of culture, the rate of ammonia metabolism was calculated. Finally, hepatocytes were also cultured with the effective supernatants (Co-cul sup (E)) with and without the combined 3 above antibodies (anti-Mix group, \( n = 8 \)).
**S5. Fluorescent staining of hepatocytes and ADSCs**

To trace the transplanted hepatocytes and ADSCs in the liver after intraportal transplantation, hepatocytes and ADSCs were labeled using CellBrite Cytoplasmic Membrane Dyes (Biotium Inc, Fremont, CA, USA). Before transplantation, hepatocytes were suspended at a density of $2.0 \times 10^5$ cells/ml and ADSCs were suspended at a density of $1.0 \times 10^6$ cells/ml, and 5 μl of CellBrite was added per 1 ml of each cell suspension. Livers were retrieved 2 days (Day 2 group, $n = 3$) and 7 days (Day 7 group, $n = 3$) after intraportal transplantation. Tissues were fixed with 4% paraformaldehyde, frozen in liquid nitrogen for frozen sections, and slides were stained with DAPI (Vibrance Antifade Mounting Medium with DAPI; Vector Laboratories, Inc.). Nine slides per rat were prepared and the number of transplanted hepatocytes and ADSC-adhered hepatocytes were counted. The rate of ADSC-adhered hepatocytes was calculated.

To detect transplanted hepatocytes and ADSCs in the liver after intraportal transplantation, these cells were labeled by fluorescent staining prior to transplantation. The micrograph showed transplanted hepatocytes (green) with adherent ADSCs (red) (see below).
Interestingly, the rate of ADSC-adhered hepatocytes at 7 days after transplantation (61.4±5.5%) was significantly higher than that at 2 days after transplantation (46.2±3.3%) (p<0.05).
