Injury Resistance in the Setting of Liver Fibrosis is Accompanied by the Inhibition of HMGB1 Translocation and Release

Supplementary information

Immunofluorescence (IF) studies

Liver tissue samples were snap frozen in liquid nitrogen and embedded in Tissue-Tek OCT compound. The liver sections were fixed and stained with the following primary antibodies: goat anti-mouse type I collagen (Col-1) monoclonal antibody (1:200; Southern Biotech, CA, USA), FITC-anti-mouse smooth muscle actin (SMA) monoclonal antibody (1:500; Sigma, St Louis, MO, USA), PE-Cy5-anti-mouse F4/80 (1:100; eBioscience, San Diego, CA), FITC-anti-mouse F4/80 (1:100; eBioscience, San Diego, CA), and rabbit anti-mouse high-mobility group box-1 (HMGB1) monoclonal antibody (1:100; Abcam, Cambridge, MA, USA). As an indirect IF staining for HMGB1 and Col-1, FITC-conjugated donkey anti-rabbit IgG (1:500; Sigma, St Louis, MO, USA), Cy3-conjugated rabbit anti-goat IgG (1:500; Sigma, St Louis, MO, USA), and FITC-chicken anti-goat IgG (1:500; Santa Cruz Biotechnology, Dallas, TX, USA) were used. Nikon Inverted Fluorescence Microscope ECLIPSE Ti (Nikon Corporation, Japan) and NIS-Elements F 3.0 Software were applied for image capture.

Supplementary Fig. 1 Mice were injected intraperitoneally with CCl_4, twice a week, for 6 weeks. Liver fibrosis was verified by Masson staining (1A) and

immunofluorescence analysis for alpha-smooth muscle actin (α-SMA) (1B). The expression of hepatic Cyp2E1 in the control and fibrotic mice with or without acute insult was determined by RT-PCR analysis (1C).

Supplementary Fig. 2 Mice were injected intraperitoneally with CCl_4, twice a week, for 6 weeks. By the end of fibrosis induction, CCl_4 administration was stopped to achieve spontaneous regression. The observation points were set at Day 6 and Day 12 during the regression phase, namely, R6d and R12d. Fibrosis regression was verified by immunofluorescence analysis for type I collagen (Col-1) (upper) and Masson staining (lower).

Supplementary Fig. 3 The intimate correlation between HMGB1 and Kupffer cells in the fibrotic liver. (A) F4/80, a surrogate marker of Kupffer cell, was co-localized with alpha-smooth muscle actin (α-SMA). (B) Immunofluroscent staining of frozen sections was conducted to determine the co-localization of high-mobility group box-1 (HMGB1), F4/80, and type I collagen (Col-1).