**Materials and Methods**

***In vivo* knockdown of SAA1 using RNAi technology**

For *in vivo* knockdown of SAA1, invivofectamine3.0-siRNA complex was prepared and injected in to C57BL/6 mice according to the manufacturer instruction (Life science technology).

siRNA sequences

SAA1-1: 5’CAACUAUGAUGCUGCUCAA3’

SAA1-2: 5’GUCCUCCUAUUAGCUCAGU3’

SAA1-3: 5’GAGGAGAGGGUAAUAAACA3’

Briefly, equal amount of 250 μl of siRNA targeting SAA1 (3 mg/ml) and complexation buffer was mixed by vortexing to form invivofectamine3.0-SAAsiRNA complex. The invivofectamine3.0-SAAsiRNA complex incubated at 50 0C for about 30 min. The complex centrifuged for 1hr at 3,000 x g (LABOGENE) and adjusted to 0.5 ml to make the final concentration 1.4mg/ml. Finally the mice injected buffer (control), NC siRNA (non-specific siRNA) or SAA1-siRNA complex (targeting SAA1) in to the lateral caudal tail vein once at day −1 before CCl4 treatment.

**Histological analysis**

Mice were sacrificed after 24 hr of CCl4 injection and the livers were fixed in 4 % paraformaldehyde and embedded in paraffin. The tissue sections (5 μm) in size were stained with H&E staining kit (Beyotime Biotech Inc.) or TUNEL using the DeadEnd Flourometric TUNEL System (Promega, G7360).

**Cell isolation and culture**

Tissues were obtained by qualified medical staff, with written donor consent and the approval of the Ethics Committee according to the Declaration of Helsinki. Primary human hepatocytes were isolated by using collagenase digestion from surgical specimens of healthy human livers as described previously [[19](#_ENREF_19)]. In brief, human liver spacemen was digested by using 0.5 % collagenase solution supplemented with calcium for 30 min. Liver was homogenized with 0.025 % collagenase for about 30 min at 37 0C. The cells suspension was filtered, centrifuged for 2 min at 50 x g and hepatocytes were isolated from the pellets. Viable hepatocytes were screened by trypan blue exclusion dye as well as by attachment to the collagen coated cell culture plates in DMEM (Thermo Fisher Scientific) supplemented with 15 % FBS (Gibco, Thermo Fisher Scientific) and 1 % Penicillin-Streptomycin (Thermo Fisher Scientific).

**Determination of cell death**

Primary human hepatocytes were treated with recombinant human SAA1 (PeproTech) 10 μg/ml or tumor necrosis factor (TNF)-α 20 ng/ml (R&D system) with or without ActD (0.2 μg/ml, Sigma) or SC75741 (50μM) (Selleck) after 12 hr serum starvation. Cell death in hepatocytes was measured by LDH release into the culture medium according to the instruction provided by the manufacturer (Thermoscientific). Caspase 3- and PARP cleavage were determined by western blot analysis. Caspase-3 activity was measured using the Caspase-3 Fluorometric Assay Kit (Biovision, USA) according to instruction provided by the manufacturer.

**Annexin V/propiduim iodide (PI) staining**

Cells were seeded into 6-well plates and cultured for 24 hr. Then Then, 0.25 % trypsin without EDTA was added to digest cells at 37 0C. the cell suspension was washed twice with clod phosphate buffered saline (PBS) and stained with 5 μL of Annexin V-fluorescein isothiocyanate (FITC)/Prodium Iodide (PI) Kit (BD Bioscience, USA) for 15 min and 5 mL of PI from 5 min at 25 0C. The cell apoptosis was assessed using (C6 Plus, BD, USA).

**Luciferase reporter gene assay**

Primary human hepatocytes were transfected with 2 μg/well of reporter plasmid for nuclear factor pGL4.32[luc2P/NF-κB-RE/Hygro] Vector (Promega cat# E849A) using Lipofectamine 3000 (Invitrogen) according to the manufacturer instructions. In all cases, 0.2 ng/well pRL wild-type *Renilla* luciferase (Rluc) control reporter vector (pGL4.75 [hRluc/CMV] Renilla luciferasecat Promega #E2311) was used. After 24 hr of incubation, the cells were stimulated by rhSAA1 (PeproTech (5 – 20 μg/ml) or rhTNF-α (20 ng) for 6 hr. Then, the reporter gene activity was measured by using Dual Luciferase Assay System (Promega) based on instructions provided by manufacturer.

**Quantitative RT-PCR**

Total RNA was extracted by using TRIzol reagent (Invitrogen) as described previously [[20](#_ENREF_20)] and, cDNA synthesis was carried out by using a revers transcription kit (TOYOBO CO., LTD). Quantitative PCR was performed using SYBR green reagent (Thermo scientific) with GAPDH as internal control. The relative standard curve method was used for quantification of gene expression in each sample. All sequences of the primers are described in Table 1.

**Table 1. Primers**

|  |  |  |
| --- | --- | --- |
| Gene | species | Sequences |
| CCL2 | Human | Forward | CAGCCAGATGCAATCAATGCC |
| Revers | TGGAATCCTGAACCCACTTCT |
| CCL5 | Human | Forward | CCAGCAGTCGTCTTTGTCAC  |
| Revers | CTCTGGGTTGGCACACACTT  |
| Eotaxin | Human | Forward | AGGATGGGCACAGAACTTCG |
| Revers | TCTTGCTTTTCAGCCCTCTTCA |
| CXCL8 | Human | Forward | CCCAGAAAGCTGTGATCTTCAA |
| Revers | GAACATTGCCCACACGTGAC |
| CXCL2 | Human | Forward | CCAACCACCAGGCTACAGG |
| Revers | GCGTCACACTCAAGCTCTG |

**Western blot analysis**

Protein extracted from primary human hepatocyte was run on 10 % SDS acrylamide gel and the gels were transferred on to the PVDF membrane (Millipore, USA) and subjected to blocking with 5% dry milk or bovine serum albumin. Blots were incubated with antibodies such as anti-caspase-3 (Cell Signaling Technology), anti-PARP (abcam), anti-GAPDH (Proteintech), MCP-1 (R&D system), Phospho-Akt and Akt (Cell signaling), Phospho-PI3K and PI3K (Cell signaling), RANTES (R&D system), Phospho-p65 (Cell signaling) anti-SAA1 (abcam) & (R&D system), 2 hr RT or overnight at 4 0C. After incubation with secondary horse anti-Rabbit or Goat anti-Mouse antibodies (abcam), the bands were developed using substrate (Thermo Scientific). The signal was detected using immobilonTM Western Chemiluminescent HRP Substrate (Millipore) and visualized by the enhanced chemiluminescence light method Tanon image system (Shanghai, China).