Interleukin-6 Mediates Angiotensinogen Gene Expression during Liver Regeneration

Hong-Shiee Lai*, Wen-Hsi Lin, Shuo-Lun Lai, Hao-Yu Lin, Wen-Ming Hsu, Chia-Hung Chou, Po-Huang Lee

Department of Surgery, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

Abstract

Background: Angiotensinogen is the precursor of angiotensin II, which is associated with ischemia-reperfusion injury. Angiotensin II reduces liver regeneration after hepatectomy and causes dysfunction and failure of reduced-size liver transplants. However, the regulation of angiotensinogen during liver regeneration is still unclear.

Aims: To investigate the regulation of angiotensinogen during liver regeneration for preventing angiotensin II-related ischemia-reperfusion injury during liver regeneration.

Methods: A mouse in vitro partial hepatectomy animal model was used to evaluate the expression of interleukin-6 (IL-6) and angiotensinogen during liver regeneration. Serum IL-6 and angiotensinogen were detected by enzyme immunoassay (EIA). Angiotensinogen mRNA was detected by RT-PCR. Tissue levels of angiotensinogen protein were detected by Western blot analysis. Primary cultures of mouse hepatocytes were used to investigate IL-6-induced angiotensinogen. Chemical inhibitors were used to perturb signal transduction pathways. Synthetic double-stranded oligodeoxynucleotides (ODNs) were used as ‘decoy’ cis-elements to investigate transcription. Ki 67 staining and quantification were used to verify liver regeneration.

Results: In the in vivo model, the levels of serum IL-6 and angiotensinogen correlated. In the in vitro model, IL-6 transcriptionally regulated angiotensinogen expression. Additionally, IL-6 mediated angiotensinogen expression through the Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) and JAK/p38 signaling. Decoy ODN analyses revealed that STAT3 and nuclear factor-kB (NF-kB) also played critical roles in the transcriptional regulation of angiotensinogen by IL-6. IL-6-mediated signaling, JAK2, STAT3 and p38 inhibitors reduced angiotensinogen expression in the partially hepatectomized mice.

Conclusion: During liver regeneration, IL-6-enhanced angiotensinogen expression is dependent on the JAK/STAT3 and JAK/p38/NF-kB signaling pathways. Interruption of the molecular mechanisms of angiotensinogen regulation may be applied as the basis of therapeutic strategies for preventing angiotensin II-related ischemia-reperfusion injury during liver regeneration.

Introduction

Liver regeneration occurs after a loss of liver mass or liver injury, such as that resulting from the resection of liver tumors or trauma repair [1,2]. Liver regeneration is widely studied by mimicking such clinical conditions via partial hepatectomy in rodents. The reproducibility of partial hepatectomy has made it the preferred approach for studies of liver regeneration. In rats and mice, partial hepatectomy triggers a sequence of regeneration events from the first 30 minutes to 7 days after the procedure. A large number of genes with either new or increased expression in the regenerating liver have been identified and categorized into several phases of activity, including immediate-early, delayed, cell cycle, and DNA replication and mitosis genes [3,4].

Angiogenesis, an important process for tissue growth, is also essential for liver regeneration [5]. During liver regeneration, several factors are involved in angiogenesis including plasminogen, vascular endothelial growth factor, and vascular endothelial cells [6–8]. On the other hand, apoptosis, a common form of cell death, occurs under both physiological and pathological conditions. Apoptosis and cell proliferation are complementary and account for the maintenance, growth, or degradation of a tissue. The regulation of apoptosis is important during liver regeneration [9,10], and there is a fine balance between cell proliferation and apoptosis. Interleukin-6 (IL-6) is an important cytokine involved in liver regeneration. Hepatocyte DNA synthesis during liver regeneration is suppressed in mice carrying a homozygous deletion of the IL-6 gene [11]. Moreover, IL-6 plays a crucial role in

* E-mail: hslai@ntu.edu.tw

Copyright: © 2013 Lai et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was supported by Taiwan National Science Council grants NSC 96-2314-B-002-079-MY3 and NSC 100-2314-B-002-019-MY3, and National Taiwan University Hospital grants NTUH 97-S883 and NTUH 100-S1491. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.
regulating the regeneration of hepatocytes after hepatitis or partial hepatectomy [12].

Angiotensinogen, an essential member in the rennin-angiotensin system, is responsible for hypertension [13,14], and angiotensinogen is also associated with liver cirrhosis, portal hypertension, and hepatic ischemia/reperfusion injury [15,16]. Interestingly, angiotensinogen is related to both angiogenesis and apoptosis. Angiotensinogen inhibits angiogenesis by inducing apoptosis of endothelial cells [17], and it also enhances apoptosis of other cell types, including alveolar cells, cardiomyocytes, and renal tubular cells [18–20]. This evidence suggests that angiotensinogen plays an essential role in the process of liver regeneration. Angiotensinogen serves as a reservoir for angiotensin I, which is cleaved from the N-terminal by the enzyme renin and is then converted into angiotensin II. Angiotensin II-related ischemia-reperfusion reduces liver regeneration after hepatectomy and is also a cause of dysfunction and failure of reduced-size liver transplants [21]. In this study, we defined the molecular regulatory effects of IL-6 on angiotensinogen during liver regeneration after partial hepatectomy through the Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) or JAK/p38/NF-kB signaling pathway.

Materials and Methods

Animals

Male imprinting control region (ICR) mice (purchased from Charles River, Osaka, Japan) weighing approximately 30 g each were used in the experiments. All mice were randomly assigned to two groups that were subjected to either 70% partial hepatectomy or a sham operation. Mice were further divided into eight subgroups that were killed either preoperatively (0 h) or 2, 4, 6, 12, 24 hours, 3 and 7 days postoperatively. All the animal care and handling procedures and experimental protocols were approved by the Committee of Experimental Animal Management at College of Medicine, National Taiwan University.

Surgical Procedures

All mice were anesthetized by inhalation of isoflurane [2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane]. Partial hepatectomy was then performed through a midline laparotomy by aseptically extirpating the median and left lateral lobes, accounting for approximately 70% of the original liver, according to the procedure of Higgins and Anderson [22]. The removed liver sample was immediately weighed and prepared for Western blot analysis. Laparotomies with manipulation of the liver were performed as the sham operation. At sacrifice, the remnant liver was excised and weighed. The original liver weight was estimated retrospectively based on the excised liver weight after 70% PH. For each time point, the ratio of remnant liver weight to the estimated original liver weight was calculated as a percentage value.

Measurement of IL-6 and Angiotensinogen Serum Levels

The serum levels of IL-6 and angiotensinogen were determined by enzyme immunoassay (R&D Systems, Minneapolis, MN, USA.
for IL-6 and Immuno-Biological Laboratories Co., Ltd., Aramachi, JAPAN for angiotensinogen) at different time points after partial hepatectomy or the sham operation.

Angiotensinogen mRNA Expression Determination

Total RNA was isolated from remnant liver tissue using RNAzol B reagent (Invitrogen, San Diego, CA) according to the manufacturer’s instructions. cDNA was prepared from 2 µg of total RNA with random hexamer primers according to the cDNA synthesis ImProm-II protocol (Promega, Madison, USA). Specific oligonucleotide primer pairs for angiotensinogen were used in the reverse transcription-polymerase chain reaction.

Western Blotting

The tissue lysates were centrifuged at 1,000 g for 10 minutes at 4°C. Protein concentrations were measured using the Bio-Rad protein assay (Hercules, CA). Then, 50-µg protein samples were separated using SDS-PAGE, transferred onto polyvinylidene difluoride membranes and immuno-blotted with various antibodies. Results were quantified by densitometry (Bio-Rad GS-800 densitometer).

Antibodies and Chemical Inhibitors

Akt (protein kinase B) antibody, Akt phosphorylations, ERK (extracellular signal-regulated kinase), ERK phosphorylations, p38, p38 phosphorylations, c-Jun N-terminal kinase [JNK], JNK phosphorylations, epidermal growth factor receptor [EGFR], EGFR phosphorylations, STAT3, STAT3 phosphorylations, and NF-kB p65 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-β-actin antibody was purchased from Alcam (Cambridge, MA). Chemical inhibitors of LY294002, PD98059, SB203580, AG1478, AG490, and SP600125 were purchased from Sigma Chemical Company (St. Louis, MO). STAT3 inhibitor, S31–201, was purchased from Calbiochem (San Diego, CA).

Culture of Mouse Hepatocytes

The mouse hepatocytes were collected from regenerated mouse livers. The cell suspensions were layered onto 10 ml of Ficoll-Paque PLUS (GE Healthcare Lifesciences). After centrifugation at 450 × g for 15 minutes, the cells in interphase were collected. The cells were treated with 80 IU/ml hyaluronidase in 1 ml of RPMI-1640 medium (Gibco) for 10 minutes. The cells were washed and suspended in RPMI-1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 0.25 mg/ml amphotericin B. The cells were then incubated at 37°C in a humidified atmosphere with 5% CO2.

Double-stranded Oligodeoxynucleotide Decoys

This study used synthetic, double-stranded oligodeoxynuclotides (ODNs) as ‘decoy’ cis-elements to block the binding of nuclear factors to the promoter regions of targeted genes, thus inhibiting gene transactivation. The following sequences of the phosphorothioate ODN were utilized: Scramble decoy ODN: 5’-TTGCGGTACCTGAATTGCA-3’; AP-1 decoy ODN: 5’-TGCAATTGGCGAATTCGCA-3’; CRE decoy ODN: 5’-CGGGAGGTTTTCCCCCTTGC-3’; NF-kB decoy ODN: 5’-CCTTTGAGGAATTTCCCTC-3’; Sp1 decoy ODN: 5’-GACGGTGTTTGGATTTCC-3’; and STAT3 decoy ODN: 5’-CATTGCCTACTGAA-3’. For transfection of hepatocytes, the decoy or scrambled decoy was mixed with the Transfactiv transfection reagent (Promega) for 15 minutes and then incubated with the cells in a serum-free medium.

Immunohistochemical Assay

The slides were rehydrated in PBS for 15 min, and endogenous peroxidase was inhibited by exposure (10 min at room temperature) to a solution of 3% H2O2 in methanol. Samples were blocked (30 min at room temperature) using 5% nonfat milk in PBS. Slides were then incubated with Ki-67 (sc-7846, Santa Cruz Biotechnology, Inc.) http://www.scbt.com/login.html for 16 hours at 4°C. The peroxidase-conjugated secondary antibody was incubated for 30 min at room temperature, and slides were developed by immersing them in 0.06% 3,3'-diaminobenzidine tetrahydrochloride (DAKO), followed by counterstaining with Gill’s Hematoxylin. V. Proliferative activity was defined by the Ki-67 staining index (SI). Nuclear staining of Ki-67 was considered positive. The Ki-67 SI was defined as the average percentage of positive nuclei from 10 high-power fields (400x) counted.

Statistical Analysis

In this study, each experiment was performed in triplicate, and all experiments were repeated at least three times on different occasions. Data were expressed as means ± standard deviations.
Paired Student’s t-tests were used to evaluate statistically significant differences between the experimental groups and the control group in specified tests. P<0.05 was considered as the statistically significant cut-off value.

**Results**

Expression of Serum IL-6 and Angiotensinogen Levels had Similar Temporal Patterns during Liver Regeneration

We evaluated the expression of IL-6 and angiotensinogen during liver regeneration after partial hepatectomy. Serum IL-6 levels peaked at 4 hours (486±30 pg/ml), while serum angiotensinogen levels peaked at 12 hours (8.6±0.67 μg/ml) and decreased at 24 hours (6.2±0.12 μg/ml) after partial hepatectomy (Fig. 1A). The in vivo partial hepatectomy-induced liver regeneration model showed that IL-6 expression began prior to that of angiotensinogen, but both had similar temporal patterns. Angiotensinogen mRNA in the remnant liver tissue was significantly upregulated from 2 to 24 hours after partial hepatectomy (Fig. 1B). Quantitative analysis of angiotensinogen protein levels in the remnant liver tissue revealed that angiotensinogen protein expression was significantly upregulated from 6 to 12 hours after partial hepatectomy (Fig. 1C).

IL-6 induced Angiotensinogen Expression in Primary Cultures of Mouse Hepatocytes

To evaluate the induction effect of IL-6 on angiotensinogen expression, primary cultures of mouse hepatocytes were treated with 10 ng/ml of recombinant mouse IL-6. Angiotensinogen protein in the cell culture supernatant was measured at the specified times. Angiotensinogen levels were significantly higher in the IL-6-treated group than in the vehicle-treated group from 6 to 24 hours in the cell culture supernatant (Fig. 2A). To evaluate the dose response of IL-6 in inducing angiotensinogen protein expression, primary cultures of mouse hepatocytes were treated with different doses of recombinant mouse IL-6. After 24 hours of culture, angiotensinogen protein in the cell culture supernatants was measured. The data revealed that IL-6 significantly induced angiotensinogen protein expression at 1 to 100 ng/ml; the responses were also dose dependent in the range of 1 to 10 ng/ml (Fig. 2B).

**IL-6-mediated Angiotensinogen Expression is Dependent on the JAK/STAT3 and JAK/p38/NF-κB Signal Transduction Pathways**

The molecular mechanisms of IL-6-induced angiotensinogen expression are not well defined. When IL-6 binds to IL-6Rα, the resultant complex associates with the signal-transducing membrane protein gp130, thereby, inducing its dimerization and the initiation of intracellular signaling via the JAK/STAT, MAPK family and PI3K pathways. We further investigated the signal transduction pathways involved in IL-6-induced angiotensinogen expression in primary cultures of mouse hepatocytes. Western blot analysis and the quantitative results revealed that the phosphatidylinositol 3-kinase inhibitor LY294002 (50 μM) significantly inhibited IL-6-induced Akt phosphorylation; the mitogen-activated protein kinase kinase inhibitor PD98059 (50 μg/ml) significantly inhibited IL-6-induced ERK phosphorylation; the mitogen-activated protein kinase p38 inhibitor SB203580 (5 μg/ml) significantly inhibited IL-6-induced p38 phosphorylation; the c-
Jun N-terminal kinase (JNK) inhibitor SP600125 (20 μM) significantly inhibited IL-6-induced JNK phosphorylation; the signal transducer and activator of transcription 3 (STAT3) inhibitor S31–201 (10 μM) significantly inhibited IL-6-induced STAT3 phosphorylation; and the EGFR tyrosine kinase inhibitor AG1478 (20 μM) significantly inhibited IL-6-induced EGFR phosphorylation (Fig. 3A). We further investigated the inhibitory effects of different chemicals on IL-6-induced angiotensinogen protein expression. The results revealed that IL-6 significantly induced angiotensinogen protein expression (467 ± 65 pg/ml), but pretreatment with SB203580 and S31–201 significantly inhibited IL-6-induced angiotensinogen protein expression (306 ± 38 and 278 ± 32 pg/ml, respectively) (Fig. 3B). Thus, the IL-6-mediated p38 and STAT3 signaling transduction pathways were critically involved in IL-6-induced angiotensinogen expression in primary cultures of mouse hepatocytes.

STAT3 and NF-kB are Critical Transcription Factors Involved in IL-6-induced Angiotensinogen Expression in Mouse Hepatocytes

To evaluate the transcription factors involved in the regulation of angiotensinogen by IL-6, synthetic double-stranded ODNs were used as ‘decoy’ cis-elements to block the binding of nuclear factors to the promoter regions of targeted genes, thus, inhibiting gene transactivation. In the promoter region of angiotensinogen, the oligonucleotides contained the consensus binding sequences for C/EBP, AP-1, NF-kB, CRE, Sp-1, and STAT3. Primary cultures of mouse hepatocytes were pretreated with different decoy ODNs (5 μM) for 24 hours prior to IL-6 (10 ng/ml) stimulation. IL-6 significantly induced angiotensinogen expression (423 ± 38 pg/ml), while NF-kB and STAT3 decoy ODNs significantly inhibited IL-6-induced angiotensinogen expression (288 ± 29 and 292 ± 26 pg/ml, respectively) (Fig. 4A). We further clarified the effect of p38- and STAT3-mediated signaling on nuclear translocation of NF-kB and STAT3. Primary cultures of mouse hepatocytes were pretreated with the p38 inhibitor SB203580 or STAT3 inhibitor S31–201 for 1 hour prior to IL-6 stimulation; the nuclear and cytosolic proteins were collected after 1 hour. Quantitative analysis of the NF-kB p65 subunit and STAT3 revealed that p38 was significantly involved in IL-6-induced NF-kB nuclear translocation, and IL-6-induced STAT3 activation was significantly involved in STAT3 nuclear translocation. Furthermore, there was no crosstalk between IL-6-mediated p38 and STAT3 signaling (Fig. 4B). We also evaluated the role of Janus kinase (JAK) on IL-6-mediated STAT3 and p38 signaling using the JAK2-specific inhibitor AG490. Primary cultures of mouse hepatocytes were pretreated with AG490 for 1 hour prior to IL-6 stimulation. Western blot and quantitative analyses showed that JAK2 signaling was significantly involved in IL-6-activated p38 and STAT3 phosphorylation (Fig. 4C). Thus, IL-6-mediated JAK2/STAT3 and NF-kB are Critical Transcription Factors Involved in IL-6-induced Angiotensinogen Expression in Mouse Hepatocytes

Figure 4. Transcription factors and their upstream signal mediators involved in IL-6-induced angiotensinogen expression in primary cultures of mouse hepatocytes. (A) Inhibition effects of different transcription factor oligodeoxynucleotide (ODN) decoys on IL-6-induced angiotensinogen protein expression as detected by enzyme immunoassay. Comparisons are between the indicated groups. *P<0.05, n=3. (B) The effects of S31–201 and SB203580 on IL-6-induced STAT3 or NF-kB p65 subunit nuclear translocation were determined by Western blotting and quantified by calculating the ratio of n (nuclear)/c (cytosolic) proteins. The ratio in lane 1 is defined as 1. Comparisons are between lanes 2 and 3 or 4 in each group. *P<0.05, n=3. (C) Effects of AG490 on IL-6-induced phospho-p38 (p-p38) and phospho-STAT3 (p-STAT3) were determined by Western blotting and quantified by calculating the ratio of phosphorylated/non-phosphorylated proteins. The ratio in lane 1 is defined as 1. Comparisons are between lanes 2 and 3 in each group. *P<0.05, n=3. doi:10.1371/journal.pone.0067868.g004
STAT3 and JAK2/p38/NF-κB signaling were required for proper IL-6-induced angiotensinogen expression in primary cultures of mouse hepatocytes.

IL-6 Downstream Signaling JAK2, STAT3 and p38 Inhibitors Reduced Angiotensinogen Expression in the Partially Hepatectomized Mice

The plasma IL-6 concentration was reported to significantly and similarly associate with the systolic (SBP) and diastolic (DBP) blood pressure [23]. Furthermore, angiotensin II-associated hypertension was attenuated in interleukin-6 knockout mice [24]. However, the effects of IL-6 downstream signaling JAK2, STAT3 and p38 inhibitors on the in vivo, angiotensinogen expression and blood pressure in the partially hepatectomized mice are not clear. To confirm the effects of IL-6-mediated signaling on angiotensinogen expression in vivo, mice were pretreated with chemical inhibitors of JAK2 (AG490, 10 mg/kg subcutaneously), p38 (intraperitoneal SB203580, 15 mg/kg), and STAT3 (intraperitoneal 5,15-DPP, 15 mg/kg) for 4 hours prior to partial hepatectomy. The results revealed that all three chemical inhibitors significantly inhibited the expression of angiotensinogen from 12 to 24 hours after partial hepatectomy (Fig. 5A). To analyze other effects of these chemical inhibitors on liver regeneration, experimental data of the remnant liver revealed no significant inhibitory effect on liver regeneration (Fig. 5B) in 24 hours after partial hepatectomy; the effect was also confirmed by detecting the proliferation of hepatocytes by ki-67 staining (Fig. 5C). Furthermore, quantification of ki-67 staining (Fig. 5D) revealed that all three chemical inhibitors did not significantly inhibit liver regeneration after partial hepatectomy. These results demonstrate that IL-6-mediated signaling contributes to systemic angiotensinogen levels during liver regeneration after partial hepatectomy in the mouse model.

Discussion

The phenomenon of liver regeneration after hepatic injury is unique [25–27]. Regulation of gene activation plays an important role in liver regeneration, and DNA synthesis occurs as early as 12 to 20 hours after partial hepatectomy. With the advent of cDNA microarray technology, which enables the screening of the...
Genome-wide expression of thousands of genes, we were able to identify increased expression of angiotensinogen immediately after partial hepatectomy, with peaks at 6 hours and silencing after 24 hours. A recent study of liver regeneration using cDNA microarrays also demonstrated a 5.0-fold increment in angiotensinogen gene expression [29]. IL-6 is critically involved in liver regeneration after partial hepatectomy; however, the role of IL-6 during liver regeneration is not well defined. Aldeniger et al. characterized a paracrine mechanism by which cells of bone marrow origin, most likely Kupffer cells, regulated the regenerative capacity of hepatocytes through IL-6 expression [29]. Gewiese–Rabsch et al. demonstrated that IL-6 trans-signaling via soluble IL-6R was important for the physiological response of the liver to CCH-induced chemical damage [26]. Kawasaki et al. reported that platelets promoted liver-specific endothelium cell proliferation and induced IL-6 and vascular endothelial growth factor production. IL-6 from liver-specific endothelium cells induced the proliferation of parenchymal hepatocytes [30]. We found that recombinant mouse IL-6 directly interacted with hepatocytes. The signaling pathways involved in angiotensinogen regulation were JAK/STAT3 and JAK/p38/NF-κB. Ray et al. hepatocytes. The signaling pathways involved in angiotensinogen found that recombinant mouse IL-6 directly interacted with soluble IL-6R was important for the physiological response of the liver to CCH-induced chemical damage [26]. Kawasaki et al. reported that platelets promoted liver-specific endothelium cell proliferation and induced IL-6 and vascular endothelial growth factor production. IL-6 from liver-specific endothelium cells induced the proliferation of parenchymal hepatocytes [30]. We found that recombinant mouse IL-6 directly interacted with hepatocytes. The signaling pathways involved in angiotensinogen regulation were JAK/STAT3 and JAK/p38/NF-κB. Ray et al. demonstrated that STAT3’s NH2-terminal acetylation was activated by the hepatic acute-phase response, and the acetylation of STAT3 was required for angiotensinogen induction by IL-6 [31].

The role of angiotensinogen in the regulation of blood pressure and water homeostasis is well recognized. However, the role of angiotensinogen in response to partial hepatectomy is not well studied. The major source of circulating angiotensinogen is the liver, and the plasma concentration of angiotensinogen is the rate-limiting step in the rennin-angiotensin system. Angiotensinogen serves as a reservoir for angiotensin I cleaved from the N-terminal by renin; angiotensin I is then converted into angiotensin II. The multifunctional effects of angiotensin II are mediated by activation of AT1 or G protein-coupled receptors [32]. In a kidney-specific angiotensinogen knockout mouse model, the presence of liver-derived angiotensinogen suggested that the mechanism of kidney disease was due to the primary source of renal angiotensinogen protein and angiotensin II [33]. The upstream regions for the regulatory motifs of angiotensinogen include acute-phase responsive elements and glucocorticoid, estrogen, cAMP, and heat shock responsive elements [34]. NF-kB expression, known to increase during liver regeneration, is also a potent inducer of angiotensinogen by binding to the acute-phase responsive elements of the angiotensinogen promoter [35]. Within 1 hour of partial hepatectomy, there is evidence of NF-kB activation, which regulates signaling in cell cycle events, inflammation, and apoptosis [36]. Our results indicate that the activation of angiotensinogen occurs in the phase of early-delayed gene activation. It is plausible that angiotensinogen expression increases after NF-kB activation. In addition, the activation of NF-kB depends on tumor necrosis factor-α (TNF-α) during liver regeneration [1,2]. TNF-α, a key regulator of liver regeneration, plays a dual role by inducing either hepatocyte proliferation or apoptosis during regeneration. TNF-α induction of cell apoptosis requires de novo angiotensin II generation [20]. Additionally, TNF-α may crosstalk with the rennin-angiotensin system in hepatocytes by enhancing the expression of key genes in the rennin-angiotensin system, including angiotensinogen [33]. These lines of evidence support the notion that the angiotensinogen signaling pathway plays an important role in TNF-α/NF-kB-mediated liver regeneration.

In conclusion, we found that during liver regeneration, IL-6-enhanced angiotensinogen expression was dependent on the JAK/STAT3 and JAK/p38/NF-κB signaling pathways. The molecular mechanisms of angiotensinogen regulation may be targeted for therapeutic strategies preventing angiotensin II-related, ischemia-reperfusion injury of liver regeneration. Nevertheless, the exact role of angiotensinogen in liver regeneration requires further study.

Author Contributions
Conceived and designed the experiments: HSL. Performed the experiments: CHC. Wrote the paper: HSL. CHC.

References
1. Fausto N (2000) Liver regeneration. J Hepatol 32: 19–31.
2. Gilgenkrantz H, Collin de l’Hortet A (2011) New insights into liver regeneration. Clin Res Hepatol Gastroenterol 35: 623–629.
3. Togo S, Makino H, Kobayashi T, Morita T, Shimizu T, et al. (2004) Mechanism of liver regeneration after partial hepatectomy using mouse cDNA microarray. J Hepatol 40: 464–471.
4. Lai HS, Chen Y, Liu WH, Chen CN, Wu HC, et al. (2005) Quantitative gene expressions analysis by cDNA microarray during liver regeneration after partial hepatectomy in rats. Surg Today 35: 396–403.
5. Drider TA, Vogten MJ, Rischm ED, van Vroonhoven TJ, Gebbink MF, et al. (2002) Liver regeneration is an angiogenesis-associated phenomenon. Ann Surg 236: 705–711.
6. Drider TA, Vogten MJ, Gebbink MF, Carmellet P, Voest EE, et al. (2003) Plasminogen mediates liver regeneration and angiogenesis after experimental partial hepatectomy. Br J Surg 90: 1384–1390.
7. LeCourtier J, Moritz DR, Li B, Phillips GL, Liang XH, et al. (2003) Angiogenesis-independent endothelial protection of liver: role of VEGFR-1. Science 309: 1890–1893.
8. Greene AK, Wiener S, Puder M, Yoshida A, Shi B, et al. (2003) Endothelial-directed hepatic regeneration after partial hepatectomy. Ann Surg 237: 530–535.
9. Wecker A, Boerger R, Vick B, Urbanzik T, Haybacek J, et al. (2010) Hepatocyte-specific deletion of the antiapoptotic protein myeloid cell leukemia-1 triggers proliferation and hepatocarcinogenesis in mice. Hepatology 51: 1226–1236.
10. Xi X, Fang JH, Yun JP, Yang J, Zhang Y, et al. (2010) Effects of microRNA-20 on apoptosis, tumorigenesis, and prognosis of hepatocellular carcinoma. Hepatology 51: 836–845.
11. Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Firth EE, et al. (1996) Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. Science 274: 1379–1383.
12. Kuma S, Inaba M, Ogata H, Inaba K, Okumura T, et al. (1990) Effect of human recombinant interleukin-6 on the proliferation of mouse hepatocytes in the primary culture. Immunobiology 180: 235–242.
13. Sun Z, Cade R, Zhang Z, Alsouder J, Van H (2003) Angiotensinogen gene knockout delays and attenuates cold-induced hypertension. Hypertension 41: 322–327.
14. Peng J, Kimura B, Phillips MI (2002) The predominant role of brain angiotensinogen and angiotensin in environmentally induced hypertension. Regul Pept 110: 25–32.
15. Sookoian S, Castaño G, García SI, Viudez P, González C, et al. (2005) A1166C angiotensin II type I receptor gene polymorphism may predict hemodynamic response to losartan in patients with cirrhosis and portal hypertension. Am J Gastroenterol 100: 656–642.
16. Luo L, Richardson KS, Tucker LM, Doll MA, Hein DW, et al. (2004) Role of the renin-angiotensin system in hepatic ischemia reperfusion injury in rats. Hepatology 40: 503–509.
17. Brand M, Lammadi N, Larger E, Corvol P, Gaar JM (2007) Angiotensinogen impairs angiogenesis in the chick chorioallantoic membrane. J Mol Med 85: 451–460.
18. Liu F, Breznicesan ML, Wei CC, Cheiner I, Sachetti S, et al. (2008) Overexpression of angiotensinogen increases tubular apoptosis in diabetes. J Am Soc Nephrol 19: 269–280.
19. Singh VP, Le B, Khodé R, Baker KM, Kumar R (2008) Intracellular angiotensin II production in diabetic rats is correlated with cardiac myocyte apoptosis, oxidative stress, and cardiac fibrosis. Diabetes 57: 3397–3406.
20. Wang R, Alam G, Zagariya A, Gidea C, Pinillos H, et al. (2000) Apoptosis of the renin-angiotensin system in hepatic ischemia reperfusion injury in rats. Hepatology 40: 503–509.
21. Padrissa Alteís S, Franco Gou R, Boillot O, Serafín A, Rimola A, et al. (2009) Directed hepatic regeneration after partial hepatectomy. Ann Surg 237: 530–535.
22. Higgins GM, Anderson RM (1931) Experimental pathology of the liver. Arch Pathol 12: 186–192.
23. Fernandez Real JM, Vayreda M, Richart C, Gutierrez C, Broch M, et al. (2001) Circulating interleukin 6 levels, blood pressure, and insulin sensitivity in apparently healthy men and women. J Clin Endocrinol Metab 86: 1154–1159.
24. Lee DL, Sturgis LC, Labazi H, Osborne JR Jr, Fleming C, et al. (2006) Angiotensin II hypertension is attenuated in interleukin-6 knockout mice. Am J Physiol Heart Circ Physiol 290: H935-H940.
25. Lai HS, Lin WH, Hsu WM, Chen CN, Chang KJ, et al. (2009) Variations in interferon gamma receptor gene expression during liver regeneration after partial hepatectomy in rats. Am Surg 75: 49–54.
26. Gewiese Rabach J, Drucker C, Malchow S, Scheller J, Rose John S (2010) Role of IL-6 trans-signaling in CCl(4) induced liver damage. Biochim Biophys Acta. 1802: 1054–1061.
27. Shanmukappa K, Sabla GE, Deon JI, Bezerra JA (2006) Urokinase-type plasminogen activator supports liver repair independent of its cellular receptor. BMC Gastroenterol 6: 40–49.
28. Xu CS, Shao HY, Liu SS, Qin B, Sun XF, et al. (2009) Possible regulation of genes associated with intracellular signaling cascade in rat liver regeneration. Scand J Gastroenterol 44: 462–470.
29. Aldeguer X, Debonera F, Shaked A, Krasinkas AM, Gelman AE, et al. (2002) Interleukin-6 from intrahepatic cells of bone marrow origin is required for normal murine liver regeneration. Hepatology 35: 40–48.
30. Kawasaki T, Murata S, Takahashi K, Nozaki R, Ohshiro Y, et al. (2010) Activation of human liver sinusoidal endothelial cell by human platelets induces hepatocyte proliferation. J Hepatol 53: 648–654.
31. Ray S, Boddorg I, Brasier AR (2003) STAT3 NH2-terminal acetylation is activated by the hepatic acute-phase response and required for IL-6 induction of angiotensinogen. Gastroenterology 129: 1616–1632.
32. Miura S, Kaya Y, Hanawa H, Nakao N, Fujino M, et al. (2012) Small molecules with similar structures exhibit agonist, neutral antagonist or inverse agonist activity toward angiotensin II type 1 receptor. PLoS One 7: e57974.
33. Matusaka T, Nisimura F, Shimizu A, Pastan I, Saito A, et al. (2012) Liver angiotensinogen is the primary source of renal angiotensin II. J Am Soc Nephrol 23: 1181–1189.
34. Fyhrquist F, Saijonmaa O (2008) Renin-angiotensin system revisited. J Intern Med 264: 224–236.
35. Fukamizu A, Takahashi S, Seo MS, Tada M, Tanimoto K, et al. (1990) Structure and expression of the human angiotensinogen gene. Identification of a unique and highly active promoter. J Biol Chem 265: 7526–7532.
36. Cresman DE, Greenbaum LE, Haber RA, Taub R (1994) Rapid activation of post-hepatectomy factor/nuclear factor kappa B in hepatocytes, a primary response in the regenerating liver. J Biol Chem 269: 30429–30435.