Original Paper

Lipocalin 2 Upregulation Protects Hepatocytes from IL1- β -Induced Stress

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Key Words

IL-1 β • IL-6 • TNF- α • LCN2 • Hepatocytes

Abstract

Background: Lipocalin 2 (LCN2), a protein primarily produced by hepatocytes, is highly upregulated under various conditions that induce cellular stress, such as intoxication, infection or inflammation. However, the precise biological functions and underlying mechanisms of LCN2 in hepatocytes remains unknown. **Methods:** Hepatocyte stress was successfully induced by treating Huh7 cells with interleukin-1 β (IL-1 β). Interleukin-6 (IL-6), Tumor Necrosis Factor- α (TNF- α) and LCN2 levels were measured in IL-1 β treated Huh7 cells and supernatant. Additionally, microarray analysis was conducted to identify genes differentially expressed in LCN2-silenced and control Huh7 cells. **Results:** TNF- α , IL-6 and LCN2 were significantly elevated in Huh7 cells after IL-1 β treatment. In LCN2-silenced Huh7 cells, expression of IL-6 and TNF- α was significantly increased when compared with the expression levels of control Huh7 cells. Furthermore, differentially expressed genes were observed between the LCN2-silenced and control cells. Microarray analysis indicated that LCN2 acted by influencing genes involved in protein metabolism, stress response, cell cycle and proliferation. **Conclusions:** Our results suggest that LCN2 upregulation protects hepatocytes from IL-1 β -induced stress. Additionally, our microarray analysis of LCN2-silenced and control cells provides a better understanding of the mechanisms that may be influenced by LCN2 induction.

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Introduction

The liver is an important immunological organ that responds quickly to bacterial infection, toxins and other sources of damaging cellular stress. Hepatic immune cells,

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including Kupffer, dendritic, NK and NK T cells, constitute the bodies first line of defense against infection [1]. However, many of the cytokines produced by hepatic immune cells can cause hepatocyte injury, and IL-1 β , IL-6 and TNF- α are especially dangerous [2, 3]. Furthermore, IL-1 β has been reported to induce high levels of TNF- α [4, 5] and IL-6 [6] in hepatocytes and osteoblasts, respectively. Because 80% of the liver volume consists of hepatocytes [7], the prevention of hepatocyte injury is an important consideration when designing therapeutic strategies [2]. Hepatocytes have been demonstrated to synthesize numerous protective proteins in response to deleterious cytokine exposure [7], and one potentially protective protein whose induction has been reported in hepatocytes after cytokine exposure is Lipocalin 2 (LCN2).

LCN2, also known as neutrophil gelatinase-associated lipocalin (NGAL), is a 25-kDa secreted glycoprotein [8]. The production of LCN2 in hepatocytes has primarily been seen in response to bacterial infection or partial hepatectomy [9]. LCN2 is highly upregulated in response to intoxication, infection, inflammation and other forms of cellular stress [10-15]. Growing evidence suggests that LCN2 plays a protective role in hepatic injury [16]. Studies have reported that activation of the transcription factors STAT3 and NF- κ B are responsible for LCN2 synthesis [9, 11, 17]; however, the mechanisms underlying LCN2's induction and cellular protective role are still not fully understood. Among IL-1 β , IL-6 and TNF- α , IL-1 β is the strongest LCN2 inducer [10, 18]. In the present study, we assessed the protective effects of LCN2 on the liver, with special emphasis on the underlying molecular mechanisms, by establishing an hepatocyte IL-1 β induced stress condition.

Materials and Methods

Cell culture

Huh7 cell were grown in DMEM medium (Gibco, Life Technologies, Grand Island, NY, USA) with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT, USA), 100 U/ml penicillin and 100 μ g/ml streptomycin in a humidified environment with 5% CO₂ at 37°C.

Small Interfering RNA (siRNA) Gene Silencing

Huh7 cells were divided into the following groups: a blank group, an IL-1 β treatment (10 ng/ml) group, an LCN2-silenced (siRNA) group and a negative control group. LCN2-siRNA (100 nM) and negative control siRNA were synthesized by RiboBio (RiboBio Co. Ltd., Guangzhou, China). siRNA constructs were transfected into Huh7 cells using Lipofectamine® RNAiMAX Reagent (Invitrogen, Life Technologies) according to the manufacturer's protocol. After 24 h, IL-1 β (10 ng/ml) was added to the media, and the cells were incubated for another 24 h. After incubation with IL-1 β , the cell culture media was collected and cells were lysed for further analysis. siRNA sequences were as follows:

Sense: 5' - GAAUGCAAUUCUCAGAGAAAdTdT - 3'

Antisense: 3' - dTdTTCUUACGUUAAGAGUCUCUU - 5'

RNA isolation and real-time PCR

Total RNA was isolated from cells using Trizol (Invitrogen) according to the manufacturer's instructions [19, 20]. RNA was quantified using a GeneQuant pro RNA/DNA Calculator spectrophotometer (Amersham Biosciences, Freiburg, Germany), and then first-strand cDNA was synthesized from 1 μ g RNA in a 20 μ l reaction volume using a PrimeScript™ RT reagent Kit with gDNA Eraser (RR047A; Takara, Shiga, Japan) according to the manufacturer's instructions. β -actin was used as a control to normalize total mRNA input and confirm cDNA synthesis efficiency. LCN2, IL-6, TNF- α , *HSPA1A*, *SOC5*, *CITED2* and β -actin mRNA expression was quantified via quantitative PCR using Power SYBR Green PCR Master MIX (RR820A; Applied Biosystems, Life Technologies) in an ABI Prism 7900 (Applied Biosystems) according to the manufacturer's instructions. The primers were purchased from Sangon Biotech (Shanghai, China), and their sequences were as follows:

LCN2:

5' - AAAGACCCGCAAAAGATGTATG - 3' (sense)

5' - AACCTGGAACAAAAGTCCTGAT - 3' (anti-sense)

IL-6:

5' – ACCCCTGACCCAACCACAAAT - 3' (sense)

5' – AGCTGCGCAGAATGAGATGAGTT - 3' (anti-sense)

TNF- α :

5' – CTGTAGCCCATGTTGTAGCAAAC - 3' (sense)

5' – GCTGGTTATCTCTCAGCTCCAC - 3' (anti-sense)

HSPA1A:

5' –AGCTGGAGCAGGTGTGTAAC - 3' (sense)

5' – CAGCAATCTTGAAAGGCC - 3' (anti-sense)

SOCS5:

5' –AGAGCGCGCACCCAAG- 3' (sense)

5' –TTATAAAATCGTGACCAATAGCAGG- 3' (sense)

CITED2:

5' –GGCTGATTTAATGCCTGAAGACT- 3' (sense)

5' –TATGTGCTCGCCCATAGGG- 3' (sense)

β -actin:

5' – GTGGCCGAGGACTTTGATTG - 3' (sense)

5' – AGTGGGGTGGCTTTTAGGATG - 3' (anti-sense)

Western blot

Cell lysates (40 μ g) were subjected to 10% SDS-PAGE and transferred onto PVDF membranes. Membranes were incubated in blocking solution containing 5% non-fat dry milk in TBST buffer for 1 h at room temperature, followed by overnight incubation at 4°C with the following primary antibodies: anti-NGAL (1:1,000, Abcam, USA) and anti- β -actin (1:3,000, MAI BIO Technology Co, Ltd., China). Antibodies were diluted in TBST with 5% milk powder. After incubation, the membranes were washed three times in TBST for 10 min, followed by incubation in goat anti-rabbit or goat anti-mouse secondary antibody (1:3,000) for 1 h at room temperature. After incubation with the secondary antibody, the washing step was repeated. Signals were detected by chemiluminescence (ECL) and exposure to X-ray film [21, 22].

ELISA

Huh7 cells were cultured in 24-well plates. After 24 hr of pre- and post-transfection IL-1 β exposure, the cells were lysed (as described above), the supernatants were collected and the levels of IL-6 (Dakewe Biotech Company Limited, Beijing, China), TNF- α (R&D Systems, Inc., Minneapolis, MN, USA) and LCN2 protein (Ray Biotech, Inc. Norcross, GA, USA) were detected using commercial ELISA kits according to the manufacturer's instructions. Absorbance was read at 450 nm with a microtiter plate reader (Bio-Rad, Hercules, CA, USA) [21]. Standard curves prepared with various concentrations of purified recombinant IL-6, TNF- α and LCN2 were used to calculate the levels of IL-6, TNF- α and LCN2 protein.

Microarray and gene ontology (GO) analysis

Total RNA was isolated from LCN2-silenced and negative control Huh7 cells. Differential expression was detected using an Agilent gene chip (Agilent Technologies Inc. Santa Clara, CA, USA) and subsequent data analysis was done using GO-analysis. Microarray analysis of 14,900 transcripts was performed. GO-analysis classified significantly different gene expression from three different angles, biological processes, cellular components and molecular functions [23]. Within the each category, a p value < 0.05 was used as the threshold of significance.

Statistical analysis

Data were analyzed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Statistical analysis was performed using independent t -tests to evaluate differences between groups. Each *in vitro* experiment was repeated at least three times, and data were expressed as means $\pm$ SEM. p < 0.05 was considered statistically significant.

Fig. 1. The IL-1 β -induced hepatocyte injury model. Expression levels of LCN2, IL-6 and TNF- α mRNA and protein levels were significantly increased in Huh7 cell supernatant after 24 h exposure to 10 ng/ml IL-1 β . A) and B) Expression levels of LCN2 mRNA and protein in the supernatant. C) and D) Expression levels of IL-6 mRNA and protein in the supernatant. E) and F) Expression levels of TNF- α mRNA and protein in the supernatant. The results of three independent experiments are expressed as means $\pm$ SEM. The blank control was compared with the 10 ng/ml IL-1 β group, ** $p < 0.01$.

Results

Expression of LCN2, IL-6 and TNF- α were increased in IL-1 β -induced hepatocytes

When compared with the control group, the mRNA expression levels of LCN2, IL-6 and TNF- α were significantly increased in Huh7 cells after IL-1 β exposure ($p < 0.01$; Fig. 1). Furthermore, LCN2, IL-6 and TNF- α protein levels were also markedly higher after IL-1 β exposure than in the control group ($p < 0.01$).

LCN2 silencing increased IL-6 and TNF- α mRNA and protein levels

After LCN2-siRNA treatment, LCN2 mRNA and protein levels were significantly lower than in control cells ($p < 0.01$; Fig. 2). In contrast, IL-6 and TNF- α mRNA expression levels in the LCN2-silenced group were significantly elevated ($p < 0.01$; Fig. 3A). The IL-6 and TNF- α protein levels in the supernatant of the LCN2-silenced group were also significantly higher when compared with the negative controls ($p < 0.05$).

GO-analysis results

When compared with negative controls, 180 genes were upregulated and 268 genes were downregulated in the LCN2-silenced group. Biological processes related to genes with differential expression were analyzed (Fig. 4A and Table 1). Genes related to the stress response, protein metabolism, cell cycle, proliferation, developmental processes and RNA metabolism were significantly differentially expressed (Table 2).

All five differentially expressed stress response genes were upregulated. Notably, among these genes, heat shock 70 kDa protein 1a (HSPA1A) and crystallin alpha B (CRYAB) were elevated 3.6- and 6.2-fold, respectively. Nine differentially regulated genes were involved in protein metabolism: *Asb11*, *Dnajb5*, *Stk40*, *Plk2*, *Limk2*, *Bbs10*, *Abi1*, *Socs5*, *Cct8* and *Pik3ca*. Of these, the first five were upregulated and the last four were downregulated. Among the differentially regulated cell cycle and proliferation genes, four were upregulated and three

Fig. 2. LCN2 was knocked down by siRNA. A) LCN2 mRNA and B) protein levels were significantly lower in the LCN2-silenced group than in the negative control group. The results of three independent experiments are expressed as means $\pm$ SEM. The blank control was compared with 10 ng/ml IL-1 β group, ** p < 0.01.

Fig. 3. Silencing LCN2 increased the expression of IL-6 and TNF- α . The expression of IL-6 and TNF- α mRNA and protein in Huh7 cell supernatant exposed to 10 ng/ml IL-1 β for 24 h after 24 h LCN2-siRNA transfection. A) and B) Expression levels of IL-6 mRNA and protein were significantly higher in the LCN2-silenced group when compared with the negative control group. C) and D) Expression levels of TNF- α mRNA and protein in supernatant were highly enhanced. * p < 0.05, ** p < 0.01.

were downregulated. Notably, *CITED2* exhibited a 7.1-fold decline. Additionally, three RNA metabolism genes were upregulated and four were downregulated, and five developmental genes were upregulated and six were downregulated.

Differentially expressed genes were localized in the membranes (32%), nucleus (19%), mitochondrion (4%), ER/Golgi (4%) and cytoskeleton (3%; Fig. 4B). Functionally, 13% of the differentially expressed genes were related to nucleic acid binding activity and 11% to signal transduction activity. A small percentage of these genes were also related to transcription

Fig. 4. Differentially expressed genes categorized according to biological processes, cellular localization and molecular functions. Genes elevated more than two-fold were considered for further analysis, the differentially expressed genes were analyzed according to gene ontology in three dimensions: A) biological processes, B) cellular localization and C) the molecular functions.

Table 1. Summary of differentially expressed genes in thirteen GO terms

GO Term	Count	p-value	Up-count	Down-count
cell cycle and proliferation	31	0.001025	14	17
Stress response	24	0.026066	15	9
transport	33	0.545953	11	22
developmental processes	47	0.033359	22	25
RNA metabolism	51	0.010529	18	33
DNA metabolism	5	0.675099	5	0
Protein metabolism	57	0.00065	25	32
Other metabolic processes	42	0.322742	14	28
Cell organization and biogenesis	34	0.073693	18	16
cell-cell signaling	6	0.319699	3	3
Signal transduction	49	0.677046	21	28
Cell adhesion	13	0.088557	6	7
death	14	0.25941	5	9

regulatory, kinase, enzyme regulator, transporter and cytoskeletal activity. However, no molecular functions could be assigned to 56% of the differentially expressed genes (Fig. 4C). In addition, the significance of the variations found for genes, such as *HSPA1A*, *SOC5* and *CITED2*, was verified by qRT-PCR (Fig. 5).

Discussion

Hepatocyte cellular stress was successfully induced *via* IL-1 β treatment of Huh7 cells to investigate the precise biological functions and underlying mechanisms of LCN2. We demonstrated that TNF- α , IL-6 and LCN2 levels were significantly elevated in Huh7 cells after IL-1 β treatment. Additionally, after siRNA knockdown of *LCN2*, IL-6 and TNF- α levels

Table 2. Differentially expressed genes involved in protein and RNA metabolism, developmental processes, stress response, cell cycle and proliferation

Gene names				
Stress response	Up	Fold change	Down	Fold change
	Ndfip1	2.006045	Eif2s1	2.284116
	Vgf	4.842413	Fbxo31	2.199312
	Mtf1	2.264259	Masp2	2.222018
	Ppard	2.658682	Runx1	2.390595
	Mapk9	2.076285	Cat	2.154408
	Cry2	2.096746	Npm1	2.225445
	Foxo3	2.325413	Tnx	2.939686
	Hspa1a	3.603816	Proz	2.965046
	Cryab	6.206847	Mdk	2.347856
	F8	13.292180		
	Akirin	2.531604		
	Ciita	2.163245		
	Herpud	2.319134		
Protein metabolism	Up	Fold change	Down	Fold change
	Asb11	15.370724	Abi1	3.394974
	Hpn	2.242927	Eif2a	2.410745
	Psmb9	2.607259	March1	2.730984
	Dnajb5	4.862749	Cpd	2.407891
	Ube2e2	2.564038	Pmpcb	2.374353
	Csnk1d	2.111092	Prss27	2.084690
	Trib1	2.213055	Hlcs	2.583186
	Fbxo33	2.682069	Ufsp2	2.168708
	Eif5	2.444075	Pign	2.063628
	Ppp1r9b	2.109372	Pdik1l	2.718698
	Pcmt1	2.079623	Socs5	6.253598
	Eed	2.209869	Tbl1xr1	2.089313
	Mrpl16	2.424925	Cet8	3.060617
	Stk40	3.235775	Pik3r1	2.351721
	Socs7	2.103468	Rpl17	2.660059
	Pnck	2.407790	Cul2	2.759068
	Rps6ka1	2.322223	Acaca	2.137593
	Usp42	2.529919	Prpf4b	2.404165
	Limk2	4.503347	Ptpn2	2.290155
Cell cycle and proliferation	Bbs10	14.032515	Rps6ka3	2.077073
			Eif3h	2.034143
			Pik3ca	6.901063
			Wsb1	2.598258
	Up	Fold change	Down	Fold change
	Mycn	3.096117	Adm	11.463231
	Axin2	3.341531	Sf1	2.235828
	Hcfc1	2.045981	Dst	2.246388
	H2afx	2.603217	Cdc73	2.635705
	Rarg	2.573744	Kitl	2.249511
	Foxc1	2.332431	Rara	2.164372
	Cd38	2.787830	Fst	2.331148
	Mafig	2.027326	Pdgfb	2.196084
	Espl1	2.645516	Vegfa	4.090032
	Txnip	3.217735	Gnb1	2.325321
	Plk2	3.036897	Cited2	7.125572
	Calm1	2.247168	Smo	2.560381
			Ccnk	3.459275
RNA metabolism	Up	Fold change	Down	Fold change
	Zfp503	2.478194	Jdp2	2.561707
	Med26	2.488779	Rnmt	2.128601
	Mycn	3.096117	Nfil3	3.947778
	Dnajb6	2.409442	Gm14325	6.196671
	Rarg	2.573744	Srrm1	2.622486
	Foxc1	2.332431	Ewsr1	2.166625
	Polr3e	4.689774	Jmjd1c	2.216034
	Akirin2	2.531604	Nudt21	3.336175
	Cbx6	2.557107	Irf2bp2	2.804514
	Hoxc	2.984191	Sp5	2.697480
			Elac2	2.067439
			Zfp36l2	2.185453
			Id2	2.343360
			Ccnl1	2.268740
			Dcps	2.058455
			Epc1	2.280560
			Atf2	2.221728
			Zbtb20	2.470606
			Vps36	2.413733
			Crcp	2.206580
			Asxl1	2.573926
			Sertad2	3.451000
				continued

were significantly increased. Our results are supported by the results of a previous study by Erawan Borkham-Kamphorst *et al.* that reported that liver damage and the levels of pro-inflammatory cytokines in *LCN2*^{-/-} mice were significantly increased after the mice were subjected to LPS, CCl₄ and ConA treatment [16].

To investigate the mechanisms underlying *LCN2*'s hepatocyte protective role, microarray analysis of the differences between the *LCN2*-silenced and negative control groups was conducted. The resultant data indicated that the protective role of *LCN2* was tightly related to genes involved in the stress response, protein metabolism, cell cycle and proliferation.

Heat shock proteins (HSPs) are molecular chaperones produced by cells in response to various stressful conditions. They are named according to their molecular weight, and HSP family members include HSP100, HSP90, HSP70, HSP60 and small HSP (sHSP) [24]. When activated by stressful stimuli, HSPs protect against inappropriate protein interactions and misfolding [25]. In the current study, heat shock 70kDa protein 1a (HSPA1A) was significantly elevated in the *LCN2*-silenced group. HSPA1A is a member of the HSP70 family, and protects proteins from aggregation and works together with other HSPs to mediate the folding of newly translated proteins in the cytosol and organelles [26]. In our study, the increased HSP expression in the *LCN2*-silenced cells indicated that the cells were under increased stress when *LCN2* was knocked down. Supporting this conclusion, others have reported that *LCN2* plays a protective role

Gene names				
Developmental processes	Up	Fold change	Down	Fold change
	Fgf23	2.051465	Upk3a	2.044423
	Pik3r	2.351721	Tcp11	2.202922
	Bicc1	2.327328	Adra1b	3.179446
	Epor	2.221886	Kif2a	2.270265
	Tob1	2.139432	Idua	2.034779
	Robo3	4.239400	Etl4	2.586668
	Axin	3.341531		
	H2afz	2.117966		
	Cst10	2.059427		
	Scrib	2.955895		
	Shank3	2.732576		
	Hoxc6	2.984191		
	Celsr3	2.380973

Fig. 5. qRT-PCR verification of gene differential expression obtained with chip data. The differential expression of HSP1A1, SOCS5 and CITED2 was confirmed by qRT-PCR.

against cellular stress [12, 27]. In LCN2 deficient cells, HSPs were overexpressed to protect against protein denaturation and cellular stress. Therefore, LCN2 appears to act in collaboration with HSPs to protect hepatocytes from environmental insults.

The balance between protein synthesis and degradation is crucial for the maintenance of a healthy cellular environment. The accumulation of misfolded or redundant proteins causes toxicity to the cells [28]. Many of the differentially expressed genes identified by the current study, such as the suppressor of cytokine signaling 5 (SOCS5), are involved in protein metabolism. SOCS5 is a negative regulator of the JAK-STAT pathway [29–31], and was substantially decreased in LCN2-silenced cells. Recent data indicate that hepatocyte production of LCN2 is primarily regulated by the transcriptional factors STAT3 and NF- κ B [9]. IL-1 β and IL-6 are thought to enhance LCN2 levels by activating the NF- κ B and JAK-STAT pathways, respectively [9, 10]. In our experiments, IL-1 β treatment induced IL-6 expression, which may have contributed, in turn, to LCN2 elevation, indicating that IL-1 β is a strong inducer of LCN2. It was reported that STAT1 and STAT3 phosphorylation were sustained and acute liver injury was enhanced in LCN2-/- mice exposed to LPS [16]. Additionally, bacteria-mediated serum LCN2 protein elevation was significantly reduced in IL-6RHep-/- mice. These results suggest that the LCN2 protein is closely associated with the JAK-STAT pathway [9]. In brief, our data strongly suggest that LCN2 plays a regulatory role in cytokine-induced hepatocyte stress by activating SOCS5, which, in turn, negatively regulates the JAK-STAT pathway.

LCN2 is reported to act as a growth factor in multiple cell types [32]. Our data supported this view by demonstrating that LCN2 expression affected the expression of cell cycle and proliferation related genes. Although leading to some proliferation inhibitor genes upregulation, LCN2 knockdown was more associated with the downregulation of genes whose downregulation has been closely linked with cell death. Our data also indicated that CITED2 expression, which has been reported to increase G1/S cell cycle progression and inhibit cellular quiescence [33], was significantly downregulated in LCN2-silenced cells. These findings indicate that LCN2 may improve hepatocyte survival by promoting cell cycle progression and proliferation.

Conclusions

Our results suggest that LCN2 upregulation protects hepatocytes from IL-1 β -induced stress. Additionally, microarray analysis indicates that LCN2 regulates protein metabolism, stress response, cell cycle and proliferation. Further studies are needed to investigate the mechanisms accounting for the protective effect of LCN2.

Abbreviations

IL-1 β (interleukin-1 β); IL-6 (interleukin-6); TNF- α (Tumor Necrosis Factor- α); LCN2 (Lipocalin 2); HSPs (heat shock proteins).

Disclosure Statement

The authors declare that they have no conflicts of interest.

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References

- 1 Racanelli V, Rehmann B: The liver as an immunological organ. *Hepatology* 2006;43:S54-62.
- 2 Malhi H, Gores GJ: Cellular and molecular mechanisms of liver injury. *Gastroenterology* 2008;134:1641-1654.
- 3 Szabo G, Mandrekar P, Dolganiuc A: Innate immune response and hepatic inflammation. *Semin Liver Dis* 2007;27:339-350.
- 4 Yoshigai E, Hara T, Inaba H, Hashimoto I, Tanaka Y, Kaibori M, Kimura T, Okumura T, Kwon AH, Nishizawa M: Interleukin-1 β induces tumor necrosis factor- α secretion from rat hepatocytes. *Hepatol Res* 2014;44:571-583.
- 5 Oishi M, Kiyono T, Sato K, Tokuhara K, Tanaka Y, Miki H, Nakatake R, Kaibori M, Nishizawa M, Okumura T, Kon M: Pyroglutamate inhibits the induction of inducible nitric oxide synthase in interleukin-1 β -stimulated primary cultured rat hepatocytes. *Nitric Oxide* 2015;44:81-87.
- 6 Kondo A, Otsuka T, Matsushima-Nishiwaki R, Kuroyanagi G, Mizutani J, Wada I, Kozawa O, Tokuda H: Inhibition of sapk/jnk leads to enhanced il-1-induced il-6 synthesis in osteoblasts. *Arch Biochem Biophys* 2013;535:227-233.
- 7 Gao B, Jeong WI, Tian Z: Liver: An organ with predominant innate immunity. *Hepatology* 2008;47:729-736.
- 8 Kjeldsen L, Johnsen AH, Sengelov H, Borregaard N: Isolation and primary structure of ngal, a novel protein associated with human neutrophil gelatinase. *J Biol Chem* 1993;268:10425-10432.
- 9 Xu MJ, Feng D, Wu H, Wang H, Chan Y, Kolls J, Borregaard N, Porse B, Berger T, Mak TW, Cowland JB, Kong X, Gao B: Liver is the major source of elevated serum lipocalin-2 levels after bacterial infection or partial hepatectomy: A critical role for il-6/stat3. *Hepatology* 2015;61:692-702.
- 10 Borkham-Kamphorst E, Drews F, Weiskirchen R: Induction of lipocalin-2 expression in acute and chronic experimental liver injury moderated by pro-inflammatory cytokines interleukin-1 β through nuclear factor- κ B activation. *Liver Int* 2011;31:656-665.
- 11 Warszawska JM, Gawish R, Sharif O, Sigel S, Doninger B, Lakovits K, Mesteri I, Nairz M, Boon L, Spiel A, Fuhrmann V, Strobl B, Muller M, Schenk P, Weiss G, Knapp S: Lipocalin 2 deactivates macrophages and worsens pneumococcal pneumonia outcomes. *J Clin Invest* 2013;123:3363-3372.

- 12 Roudkenar MH, Kuwahara Y, Baba T, Roushandeh AM, Ebishima S, Abe S, Ohkubo Y, Fukumoto M: Oxidative stress induced lipocalin 2 gene expression: Addressing its expression under the harmful conditions. *J Radiat Res* 2007;48:39-44.
- 13 Roudkenar MH, Halabian R, Roushandeh AM, Nourani MR, Masroori N, Ebrahimi M, Nikogoftar M, Rouhbakhsh M, Bahmani P, Najafabadi AJ, Shokrgozar MA: Lipocalin 2 regulation by thermal stresses: Protective role of lcn2/ngal against cold and heat stresses. *Exp Cell Res* 2009;315:3140-3151.
- 14 Chakraborty S, Kaur S, Guha S, Batra SK: The multifaceted roles of neutrophil gelatinase associated lipocalin (ngal) in inflammation and cancer. *Biochim Biophys Acta* 2012;1826:129-169.
- 15 Chen F, He JL, Zheng M, Zhu HH, Li SP, Wang K, Zhang XX, Zhao YR, Wu SS, Chen Z: Complementary laboratory indices for predicting the disease status of patients with hepatitis b virus infection. *J Viral Hepat* 2013;20:566-574.
- 16 Borkham-Kamphorst E, de Leur Ev, Zimmermann HW, Karlmark KR, Tihaa L, Haas U, Tacke F, Berger T, Mak TW, Weiskirchen R: Protective effects of lipocalin-2 (lcn2) in acute liver injury suggest a novel function in liver homeostasis. *Biochimica Et Biophysica Acta-Mol Basis Dis* 2013;1832:660-673.
- 17 Guo H, Jin D, Chen X: Lipocalin 2 is a regulator of macrophage polarization and nf-kappab/stat3 pathway activation. *Mol Endocrinol* 2014;28:1616-1628.
- 18 Cowland JB, Muta T, Borregaard N: Il-1 β -specific up-regulation of neutrophil gelatinase-associated lipocalin is controlled by ikappab-zeta. *J Immunol* 2006;176:5559-5566.
- 19 Sola A, Weigert A, Jung M, Vinuesa E, Brecht K, Weis N, Brune B, Borregaard N, Hotter G: Sphingosine-1-phosphate signalling induces the production of lcn-2 by macrophages to promote kidney regeneration. *J Pathol* 2011;225:597-608.
- 20 Asimakopoulou A, Borkham-Kamphorst E, Henning M, Yagmur E, Gassler N, Liedtke C, Berger T, Mak TW, Weiskirchen R: Lipocalin-2 (lcn2) regulates plin5 expression and intracellular lipid droplet formation in the liver. *Biochim Biophys Acta* 2014;1842:1513-1524.
- 21 Yang Y, Li S, Yang Q, Shi Y, Zheng M, Liu Y, Chen F, Song G, Xu H, Wan T, He J, Chen Z: Resveratrol reduces the proinflammatory effects and lipopolysaccharide- induced expression of hmgb1 and tlr4 in raw264.7 cells. *Cell Physiol Biochem* 2014;33:1283-1292.
- 22 Liu WX, Zhu HH, Wu W, He JL, Chen Z: Inhibition effect produced by dominant negative mutant fusion protein pres2-tlm-scfv-hbcdn on hbv replication in vitro. *J Viral Hepat* 2012;19:295-300.
- 23 Weber B, Bader N, Lehnich H, Simm A, Silber RE, Bartling B: Microarray-based gene expression profiling suggests adaptation of lung epithelial cells subjected to chronic cyclic strain. *Cell Physiol Biochem* 2014;33:1452-1466.
- 24 Doyle SM, Genest O, Wickner S: Protein rescue from aggregates by powerful molecular chaperone machines. *Nat Rev Mol Cell Biol* 2013;14:617-629.
- 25 Bozaykut P, Ozer NK, Karademir B: Regulation of protein turnover by heat shock proteins. *Free Radical Biol Med* 2014;77:195-209.
- 26 Shorter J: The mammalian disaggregase machinery: Hsp110 synergizes with hsp70 and hsp40 to catalyze protein disaggregation and reactivation in a cell-free system. *PLoS One* 2011;6:e26319.
- 27 Roudkenar MH, Halabian R, Ghasemipour Z, Roushandeh AM, Rouhbakhsh M, Nekogoftar M, Kuwahara Y, Fukumoto M, Shokrgozar MA: Neutrophil gelatinase-associated lipocalin acts as a protective factor against h(2)o(2) toxicity. *Arch Med Res* 2008;39:560-566.
- 28 Amm I, Sommer T, Wolf DH: Protein quality control and elimination of protein waste: The role of the ubiquitin-proteasome system. *Biochim Biophys Acta* 2014;1843:182-196.
- 29 Zhuang G, Wu X, Jiang Z, Kasman I, Yao J, Guan Y, Oeh J, Modrusan Z, Bais C, Sampath D, Ferrara N: Tumour-secreted mir-9 promotes endothelial cell migration and angiogenesis by activating the jak-stat pathway. *EMBO J* 2012;31:3513-3523.
- 30 Kile BT, Schulman BA, Alexander WS, Nicola NA, Martin HM, Hilton DJ: The socs box: A tale of destruction and degradation. *Trends Biochem Sci* 2002;27:235-241.
- 31 Shuai K, Liu B: Regulation of jak-stat signalling in the immune system. *Nat Rev Immunol* 2003;3:900-911.
- 32 Schmidt-Ott KM, Mori K, Li JY, Kalandadze A, Cohen DJ, Devarajan P, Barasch J: Dual action of neutrophil gelatinase-associated lipocalin. *J Am Soc Nephrol* 2007;18:407-413.
- 33 Chou YT, Hsieh CH, Chiou SH, Hsu CF, Kao YR, Lee CC, Chung CH, Wang YH, Hsu HS, Pang ST, Shieh YS, Wu CW: Cited2 functions as a molecular switch of cytokine-induced proliferation and quiescence. *Cell Death Differ* 2012;19:2015-2028.