World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2026 January 27; 18(1): 113896

DOI: 10.4254/wjh.v18.i1.113896

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Basic Study

Transforming growth factor beta reduces proprotein convertase subtilisin/kexin type 9 in the supernatant of hepatic stellate cells

Jan Bundschuh, Maximilian Neumann, Sebastian Zimny, Marlen Spirk, Christa Buechler

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade A, Grade A, Grade A, Grade B

Novelty: Grade A, Grade A, Grade A, Grade B

Creativity or Innovation: Grade A, Grade A, Grade A, Grade B

Scientific Significance: Grade A, Grade A, Grade B, Grade B

P-Reviewer: Cao Y, PhD, Associate Professor, China; Rafaqat S, PhD, Pakistan

Received: September 8, 2025

Revised: October 10, 2025

Accepted: November 26, 2025

Published online: January 27, 2026

Processing time: 143 Days and 23.1 Hours

Jan Bundschuh, Maximilian Neumann, Sebastian Zimny, Marlen Spirk, Christa Buechler, Department of Internal Medicine I, University Hospital Regensburg, Regensburg 93053, Bavaria, Germany

Corresponding author: Christa Buechler, PhD, Associate Professor, Senior Researcher, Department of Internal Medicine I, University Hospital Regensburg, Franz-Josef-Strauss Allee 11, Regensburg 93053, Bavaria, Germany. christa.buechler@klinik.uni-regensburg.de

Abstract

BACKGROUND

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is abundantly expressed by hepatocytes and regulates the uptake of low-density lipoprotein by these parenchymal cells. Little research has been conducted on PCSK9 expression in non-hepatocyte liver cells.

AIM

To investigate PCSK9 levels in the supernatant of different liver cells and its regulation in hepatic stellate cells (HSCs).

METHODS

PCSK9 levels were measured in the cell culture medium of primary human hepatocytes, HepG2 and Huh7 cells, primary human HSCs, the HSC cell line LX-2, primary human Kupffer cells, and primary human sinusoidal endothelial cells. The effects of cytokines, adipokines, lipopolysaccharide, transforming growth factor beta (TGF-β) and ligands of nuclear receptors on PCSK9 levels in LX-2 cells during 24 hours of culture were determined using enzyme-linked immunosorbent assay.

RESULTS

Primary human hepatocytes, HepG2, Huh7, HSCs, and LX-2 cells secreted significant levels of PCSK9. There were low levels of PCSK9 in the supernatant of Kupffer cells and sinusoidal endothelial cells. Interleukin-6 reduced PCSK9 in LX-2 cells to 86% of controls and lipopolysaccharide increased it by 7%, whereas tumor necrosis factor, as well as exogenous adiponectin and leptin had no effect. Chemerin-156, but not chemerin-155 or chemerin-157 isoform overexpressed in LX-2 cells, reduced PCSK9 to 84% of the controls. TGF-β reduced PCSK9 in LX-2 cell culture media to 68% of controls and lowered its cellular level. Activation of liver X receptor but not farnesoid X receptor or peroxisome proliferator-activated

receptor gamma, reduced PCSK9 levels by 42% in LX-2 cell culture medium.

CONCLUSION
Profibrotic TGF-β and the antifibrotic liver X receptor ligand both reduced PCSK9 in LX-2 medium, showing that PCSK9 is not a marker of HSC activation.

Key Words: Hepatic stellate cells; Kupffer cells; Leptin; Lipopolysaccharide; Transforming growth factor beta; Proprotein convertase subtilisin/kexin type 9; Liver X receptor

Core Tip: This study showed that primary hepatic stellate cells and the LX-2 cell line secrete proprotein convertase subtilisin/kexin type 9 (PCSK9). Levels of PCSK9 in the medium of LX-2 cells were found to be reduced by interleukin-6, chemerin-156, and transforming growth factor beta, and increased by lipopolysaccharide. The liver X receptor agonist lowered PCSK9 levels in the cell medium. As transforming growth factor beta is a fibrotic cytokine and activation of the liver X receptor exerts antifibrotic effects, PCSK9 levels in the cell medium cannot be used as a marker of activated hepatic stellate cells.

Citation: Bundschuh J, Neumann M, Zimny S, Spirk M, Buechler C. Transforming growth factor beta reduces proprotein convertase subtilisin/kexin type 9 in the supernatant of hepatic stellate cells. *World J Hepatol* 2026; 18(1): 113896
URL: https://www.wjgnet.com/1948-5182/full/v18/i1/113896.htm
DOI: https://dx.doi.org/10.4254/wjh.v18.i1.113896

INTRODUCTION

The liver is composed of various cell types, and approximately 80% of them are hepatocytes. The remaining 20% are non-parenchymal cells, including immune cells such as Kupffer cells, dendritic cells, and T cells, as well as sinusoidal endothelial cells, biliary epithelial cells, and hepatic stellate cells (HSCs)[1]. HSCs are mesenchymal cells that reside in the space of Disse, the perisinusoidal area between endothelial cells and hepatocytes. HSCs are the main storage site for retinyl esters, which are found in cytoplasmic lipid droplets. In addition to retinyl esters, these lipid droplets contain triglycerides, free fatty acids, phospholipids, free cholesterol, and cholesteryl ester[2,3]. HSCs play a central role in fibrogenesis, and when activated, produce the extracellular matrix. During activation, these cells deplete their lipid droplets[4].

HSC activation can be achieved through various stimuli. Chronic liver disease is associated with a leaky intestinal barrier and exposure of the liver to lipopolysaccharide (LPS). HSCs express the Toll-like receptor 4, and respond to bacterial LPS[5]. In the liver, interleukin (IL)-6 can bind to the IL-6 receptor, which is located on the surface of hepatocytes and HSCs. This forms the IL-6/IL-6 receptor complex, which subsequently recruits glycoprotein 130 to initiate downstream signaling[6]. Under physiological conditions, IL-6 is involved in liver regeneration, defense against infection, and metabolic functions. However, chronic activation of the IL-6 pathway is detrimental, contributing to inflammation and fibrosis[6,7]. Tumor necrosis factor (TNF) produced by Kupffer cells increases the IL-6 secretion from these cells[6]. Both TNF and IL-6 can induce quiescent HSCs to transform into myofibroblasts[8,9].

Excess adipose tissue is a risk factor for the progression of chronic liver diseases[10,11]. IL-6 can also be considered an adipokine, as it is produced in adipose tissue and its levels increase with obesity[12]. All proteins released by adipose tissue are called adipokines and can be cytokines, chemokines, and hormones. The adipokines that are mostly produced by adipocytes are adiponectin and leptin[13,14]. Adiponectin exerts anti-inflammatory effects and reduces the proliferation of HSCs and synthesis of the extracellular matrix[15]. The adipokine leptin, which is increased in the serum of obese individuals[13,14], is a profibrotic adipokine[15]. Chemerin is expressed by adipocytes and hepatocytes, and circulates in the serum as an inactive precursor protein (chemerin-163). C-terminal cleavage of chemerin produces active isoforms, one of which is chemerin-156. The exact biological functions of the different chemerin isoforms have not been defined, and both pro- and anti-inflammatory effects have been described[16]. Overexpression of chemerin-156 in the HSC cell line LX-2 increases IL-6, IL-8, and galectin-3 in the cell supernatant, showing that this isoform activates these cells[16,17].

Transforming growth factor beta (TGF-β) is a cytokine that regulates a variety of cellular functions. It controls the immune response, inflammation, cell differentiation, and proliferation. In situations of infection or chronic injury, TGF-β signaling becomes dysregulated, and the activation of TGF-β is sufficient to induce fibrogenesis[18,19]. TGF-β activates HSCs to become myofibroblasts and enhances the proliferation of these cells and the production of extracellular matrix proteins, such as collagens[4,20]. This process is physiologically halted upon depletion of active TGF-β, a pathway that is dysfunctional in chronic liver disease[18].

LX-2 cells are a human HSC cell line that retains most of the characteristics of primary HSCs[21]. Gene expression in LX-2 cells and in primary HSCs shows 98.7% similarity, and both cell types increase procollagen messenger RNA (mRNA) expression when stimulated with TGF-β[21].

Nuclear receptors are considered therapeutic targets for different diseases. Agonists of the liver X receptor (LXR) prevent the activation of HSCs, and LXR null mice exhibit increased liver injury in experimental models[22]. A farnesoid X receptor (FXR) agonist has also been shown to protect mice against hepatic inflammation and fibrosis[23]. The downregulation of peroxisome proliferator-activated receptor gamma (PPAR-γ) in activated HSCs contributes to cell proliferation and collagen synthesis[24]. As specific agonists of these nuclear receptors are available, they can be used to treat chronic liver disease[22-24].

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is highly expressed by hepatocytes and inhibits recycling of the low-density lipoprotein (LDL) receptor. Consequently, LDL uptake by the liver is reduced and LDL levels in the blood increase[25-27]. Overexpression of PCSK9 in cardiac fibroblasts, as well as the addition of recombinant PCSK9 to the cells, activates them to become myofibroblasts with higher collagen and alpha-smooth muscle actin synthesis[28]. PCSK9 also increases the expression of nucleotide-binding domain-like receptor protein 3 and nuclear factor kappa B signaling in cardiac fibroblasts[29]. These studies showed that PCSK9 plays a role in myofibroblast activation. PCSK9 in HSCs is induced by cholesterol loading. However, feeding a hypercholesterolemic diet reduces its expression in the liver, demonstrating the differential regulation of PCSK9 in HSCs compared to parenchymal cells[30].

In HepG2 cells, TNF increases PCSK9 *via* a pathway involving suppressor of cytokine signaling-3[31]. LPS increases PCSK9 mRNA and reduces LDL receptor protein in the murine liver[32]. Leptin also induces PCSK9 levels in HepG2 cells, which are associated with lower LDL receptor levels[33,34]. Conversely, IL-1β reduces PCSK9 and increases the LDL receptor in HepG2 cells[35]. This demonstrates that pro-inflammatory stimuli have opposing effects on PCSK9 expression in HepG2 cells. Moreover, adiponectin receptor agonists, which protect from metabolic diseases and inflammation[15,36], induce PCSK9 expression and its serum levels in wild-type mice[37]. It is also worth noting that adiponectin receptor agonists upregulate the LDL receptor, ultimately reducing LDL cholesterol levels[37].

PCSK9 expression is regulated both transcriptionally and post-transcriptionally, and its secretion is regulated by unknown pathways[38]. Most circulating PCSK9, which has a half-life of about 5 minutes in rodents, is derived from hepatocytes[38]. This suggests that the protein is cleared rapidly and that continuous synthesis is needed to maintain serum PCSK9 levels. However, the expression of PCSK9 in different liver cells and how it is regulated in cells other than hepatocytes, has hardly been studied.

This study first measured PCSK9 in the supernatant of primary hepatocytes, HSCs, Kupffer cells, and sinusoidal endothelial cells, as well as in the hepatocyte cell lines Huh7 and HepG2 and the HSC line LX-2[21]. As PCSK9 was found to be abundant in LX-2 cells, this study focused on its regulation by the cytokines IL-6, TNF, and TGF-β, as well as by the adipokines leptin, adiponectin and chemerin, in these cells. The effects of PPAR-γ, LXR, and FXR ligands on PCSK9 were also studied, since these ligands inhibit the activation of HSCs[22,24].

MATERIALS AND METHODS

Ethical statement

Primary hepatocytes and supernatants for the isolation of HSCs were provided by the group of Professor Dr. Thomas Weiss (University Children's Hospital Regensburg, Regensburg, Germany). Experimental procedures were performed according to the guidelines of the charitable state-controlled foundation Human Tissue and Cell Research, with written informed patient consent approved by the local Ethical Committee of the University Hospital of Regensburg (Approval No. 12-101-0048).

Cell lines

HepG2 and Huh7 cells were obtained from the American Type Culture Collection (Manassas, VA, United States). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) high glucose (PAA Laboratories GmbH, Karlsruhe, Germany) supplemented with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin. The LX-2 cells were from Merck Chemicals GmbH (Darmstadt, Germany) and were cultured in DMEM high glucose containing 2% FCS and 1% penicillin/streptomycin. HepG2, Huh7, and LX-2 cells used in the current experiments were sub-cultured to passages 7, 8, or 9.

Primary cells

Human hepatic sinusoidal endothelial cells of three different donors were obtained from ScienCell (Carlsbad, CA, United States) and cultured according to the supplier's recommendations. The cells were cryopreserved at passage 1 and used at passage 2. Human Kupffer cells of two different donors were purchased from Thermo Fisher Scientific (Schwerte, Germany) and used after overnight culture at passage 0.

Purification of primary hepatocytes

Primary hepatocytes were isolated by the group of Prof. Dr. Thomas Weiss (University Children's Hospital Regensburg) and cultivated as previously described[39]. Hepatocytes were isolated by a modified two-step ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid/collagenase perfusion procedure[39]. Human liver tissue was obtained from liver resections performed on patients undergoing partial hepatectomy due to metastatic liver tumors associated with

colorectal cancer. Primary human hepatocytes (PHHs) were cultured in serum-free DMEM supplemented with 4.5 g/L glucose, 0.4 ng/mL hydrocortisone, 0.415 × 10^{-6} U/mL insulin, 2 mmol/L glutamine, and 100 U/mL penicillin/streptomycin[40]. The viability of the isolated hepatocytes was assessed using trypan blue exclusion, and only cells with viability greater than 85% were used for cell culture. The cells were plated onto collagen-coated 6-well plates at a density of 1 × 10^5 cells/cm^2, using an appropriate volume of culture medium, and were used after 24 hours of culture at passage 0.

Purification of primary HSCs

The supernatant, which lacked hepatocytes, was used to isolate HSCs. The supernatant was centrifuged at 50 g for 5 minutes to obtain the nonparenchymal cells. Then the cells were suspended in 10 mL of DMEM high glucose supplemented with 10% FCS and 1% penicillin/streptomycin and transferred to cell culture flasks. The medium was replaced after 90 minutes and changed daily for 4 days. After a 14-day cultivation period, human stellate cells were successfully obtained. These cells express alpha-smooth muscle actin and collagen 1a1, markers of activated HSCs[4]. A total of 40000 cells were incubated in 1 mL of medium. HSC cells were used at passage 3, 4, or 5.

Incubation of cells with LPS

LPS was purchased from Sigma (Deisenhofen, Germany). Stimulation with LPS was performed in medium containing 2% FCS. LPS was used at high concentrations (1 μg/mL and 10 μg/mL). Previously, Jeschke *et al*[40] used 10 μg/mL of LPS to activate primary human liver cells, and Chen *et al*[41] used 0.1 ng/mL, 1 ng/mL, 10 ng/mL, and 100 ng/mL for LX-2 cells. Three wells were used for each experimental setup. Mean values were used for calculations and four independent experiments were performed.

Incubation of cells with cytokines

TNF, TGF-β, and IL-6 were obtained from R&D Systems (Wiesbaden-Nordenstadt, Germany). Incubations with cytokines were performed in serum-free medium. TNF was used at concentrations of 0.2 ng/mL, 2 ng/mL, and 4 ng/mL, corresponding to TNF levels released by the livers of rats injected with LPS[42] and to serum TNF levels in patients with liver disease[43]. IL-6 was used at concentrations of 5 ng/mL, 10 ng/mL, and 20 ng/mL, which correspond to serum levels of patients with chronic liver disease[44]. TGF-β was used at concentrations of 0.2 ng/mL, 2 ng/mL, and 4 ng/mL, which are similar to serum levels in patients with liver disease[45], and the 2 ng/mL and 5 ng/mL used in recent studies[43,46]. It should be noted that the different cytokine enzyme-linked immunosorbent assays (ELISAs) are not standardized, which makes it difficult to compare absolute levels determined by different studies. Three wells were used for each experimental setup. Mean values were used for calculations and at least three independent experiments were performed.

Incubation of cells with adipokines

Recombinant human adiponectin and leptin were obtained from R&D Systems (Wiesbaden-Nordenstadt, Germany). Incubations with adiponectin and leptin were performed in serum-free medium. Adiponectin was used at a concentration of 10 μg/mL, while leptin was used at concentrations of 4 ng/mL, 10 ng/mL, and 20 ng/mL. These concentrations correspond to the serum levels of these adipokines[47,48]. Three wells were used for each experimental setup, and mean values were used for calculations. At least four independent experiments were performed.

Incubation of cells with agonists of nuclear receptors

The LXR agonist T0901317 and the FXR agonist GW4064 were purchased from Sigma (Deisenhofen, Germany). Rosiglitazone, an agonist for PPAR-γ, was from Biomol GmbH (Hamburg, Germany). LXR ligand was used at 5 μmol/L and 10 μmol/L, and up to 1 μmol/L and 10 μmol/L have been used in prior studies[49,50]. GW4064 was used at 1 μmol/L in a prior study[51], and 1 μmol/L and 2 μmol/L were used in the current experiments. PPAR-g ligand was used at 7.5 μmol/L and 15 μmol/L, similar to a previous study using 10 μmol/L[24]. Three wells were used for each experimental setup, and mean values were used for calculations. At least three independent experiments were performed.

Overexpression of chemerin isoforms in LX-2 cells

The chemerin isoform chemerin-155, chemerin-156, and chemerin-157 were overexpressed in LX-2 cells cultured in medium with 2% FCS as described in our previous study[17]. PCSK9 was measured in the cell culture medium at 24 hours post-transfection. Four independent experiments were performed.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblotting

Twenty micrograms of protein were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes (Bio-Rad, Munich, Germany). Then membranes were incubated with antibodies in a solution of 1.5% bovine serum albumin in tris(hydroxymethyl)aminomethane-buffered saline containing 0.1% Tween. Signals were detected using enhanced chemiluminescence Prime Western Blot Reagent (Amersham Pharmacia, Deisenhofen, Germany). ImageJ software (National Institutes of Health, Bethesda, MD, United States)[52] was used for signal quantification. The PCSK9 antibody (#85813), cyclophilin antibody (#2175), and GAPDH antibody (#2118) were obtained from Cell Signaling Technology (Leiden, the Netherlands).

PCSK9 ELISA

The PCSK9 ELISA Kit (No. DY3888) was from Bio-Techne in Wiesbaden-Nordenstadt, Germany. Supernatants were

analyzed without dilution. All measurements were performed twice, and the mean values were used for subsequent calculations.

Statistical analysis

Data are shown as mean ± SD. The statistical tests used were a paired *t*-test (for comparison of two groups) and a one-factor analysis of variance followed by a Fisher's Least Significant Difference *post hoc* test (for comparison of three or more different groups; IBM SPSS Statistics 26.0; IBM Corp., Armonk, NY, United States). $P < 0.05$ was considered statistically significant.

RESULTS

PCSK9 levels in the supernatants of liver cells

PCSK9 levels were determined (*via* ELISA) in the cell culture supernatant of 6 different LX-2 cell cultures, primary human HSCs from 9 different donors, PHHs from 10 different donors, 16 different Huh7 cell cultures, 5 different HepG2 cell cultures, human hepatic sinusoidal endothelial cell cultures from 3 different donors, and primary human Kupffer cell cultures from 2 different donors (Table 1). PCSK9 levels were calculated for 10^6 plated cells. As the number of primary cells does not increase and the number of cells was not determined at the end of the study, the PCSK9 levels of the cell lines may be 20%-30% lower than those of the primary cells. However, they are still much higher than those of the primary cells.

The highest levels of PCSK9 were found in HepG2 cells, followed by Huh7 cells. By contrast, Kupffer cells and hepatic sinusoidal endothelial cells exhibited very low PCSK9 levels in their supernatant (Figure 1A; Table 1). PCSK9 levels in the PHH supernatant were much lower than in the HepG2 and Huh7 cell supernatants, and also lower than that in the LX-2 cell supernatant. PCSK9 levels in PHHs and HSCs were comparable (Figure 1A; Table 1).

Immunoblot analysis of HepG2 and Huh7 cells showed much higher expression of PCSK9 in the latter cell line (Figure 1B). Immunoblots were quantified by ImageJ software and the PCSK9 protein levels were normalized by dividing them by the housekeeping protein GAPDH. PCSK9 levels were 0.14 ± 0.03 in HepG2 and 1.16 ± 0.34 in Huh7 cells ($P = 0.002$). Immunoblot analysis of PCSK9 in HepG2 and LX-2 cells showed an expression of 0.52 ± 0.33 in LX-2 cells and of 0.94 ± 0.72 in HepG2 cells, and cellular protein levels in both cell lines were similar ($P = 0.475$; Figure 1C). Here, cyclophilin A was used as a housekeeping protein, which was similarly expressed in both cell lines (Figure 1C). Quantification of immunoblots is semiquantitative, and values of different experiments cannot be compared.

PCSK9 levels of LX-2 cells during culture and the effects of LPS and TGF-β

The level of PCSK9 in the LX-2 cell culture supernatant increased during cultivation (Figure 1D). Levels increased to 216% after 48 hours and to 366% after 72 hours, compared to cells cultivated for 24 hours (Figure 1D). As the doubling time of this cell line is 3 days to 4 days[21], it can be concluded that the higher levels are not primarily due to a higher cell count. This suggests that PCSK9 is relatively stable in LX-2 cell culture medium.

Both LPS and TGF-β activate HSCs[5]. Cultivation of LX-2 cells exposed to 10 μg/mL of LPS increased PCSK9 levels by 7% compared to the controls (Figure 2A). LPS at a concentration of 1 μg/mL had no effect (Figure 2A). TGF-β reduced PCSK9 by 32% over 24 hours of culture with 7 ng/mL TGF-β. A similar effect was observed with 5 ng/mL, but this was not significant as the results were based on only three independent experiments (Figure 2B).

As the effect of TGF-β was greater than that of LPS and within the range that could be detected by immunoblot, the impact of TGF-β on cellular PCSK9 was examined. Immunoblot analysis showed downregulation of PCSK9 in LX-2 cell lysates by 5 ng/mL and 7 ng/mL TGF-b (Figure 2C).

Effect of TNF and IL-6

The levels of PCSK9 in LX-2 cell culture supernatant did not change following stimulation with 0.2 ng/mL, 2 ng/mL, or 4 ng/mL of TNF (Figure 3A). IL-6 significantly reduced PCSK9 levels to 86% of those in the control group at 20 ng/mL and showed a trend towards reducing PCSK9 levels ($P = 0.085$) at 10 ng/mL (Figure 3B). The concentration of 5 ng/mL of IL-6 had no effect (Figure 3B).

Effect of adipokines

Adiponectin is an antifibrotic adipokine, whereas leptin exhibits pro-fibrotic activity[14]. Neither of these adipokines changed the PCSK9 levels of LX-2 cells (Figure 3C and D). Adiponectin was used at a concentration of 10 μg/mL, while leptin was used at concentrations of 4 ng/mL, 10 ng/mL, and 20 ng/mL. The overexpression of chemerin-155, an inactive chemerin isoform, and chemerin-157, a highly active isoform[16], had no effect on PCSK9 levels. However, chemerin-156 lowered PCSK9 by 16% compared to the controls (Figure 3E).

Our group has previously described LX-2 cells that overexpress chemerin isoforms[17]. The concentration of total chemerin in the supernatant measured by ELISA was 0.5 ng/mL in control-transfected LX-2 cells, 8 ng/mL in chemerin-155-overexpressing cells, 3 ng/mL in chemerin-156 cells, and 9 ng/mL in chemerin-157-overexpressing cells[17]. Chemerin ELISA cannot differentiate the chemerin isoforms. Cellular PCSK9 levels were not measured due to the modest effect of chemerin-156 on soluble levels.

Table 1 Proprotein convertase subtilisin/kexin type 9 levels in supernatants of primary liver cells and cell lines, mean ± SD

Cells	LX-2	HSC	PHH	Huh7	HepG2	HHSEC	KC
Number of cultures	6	9		16	5		
Number of donors			10			3	2
PCSK9 (ng/mL)	6.8 ± 1.7	1.7 ± 1.5	2.4 ± 0.5	17.2 ± 6.9	27.0 ± 1.5	0.2 ± 0.1	0.1 ± 0.0

Proprotein convertase subtilisin/kexin type 9 protein levels of 10^6 cells are listed in the table. PCSK9: Proprotein convertase subtilisin/kexin type 9; HSC: Hepatic stellate cell; PHH: Primary human hepatic; HHSEC: Human hepatic sinusoidal endothelial cell; KC: Kupffer cell.

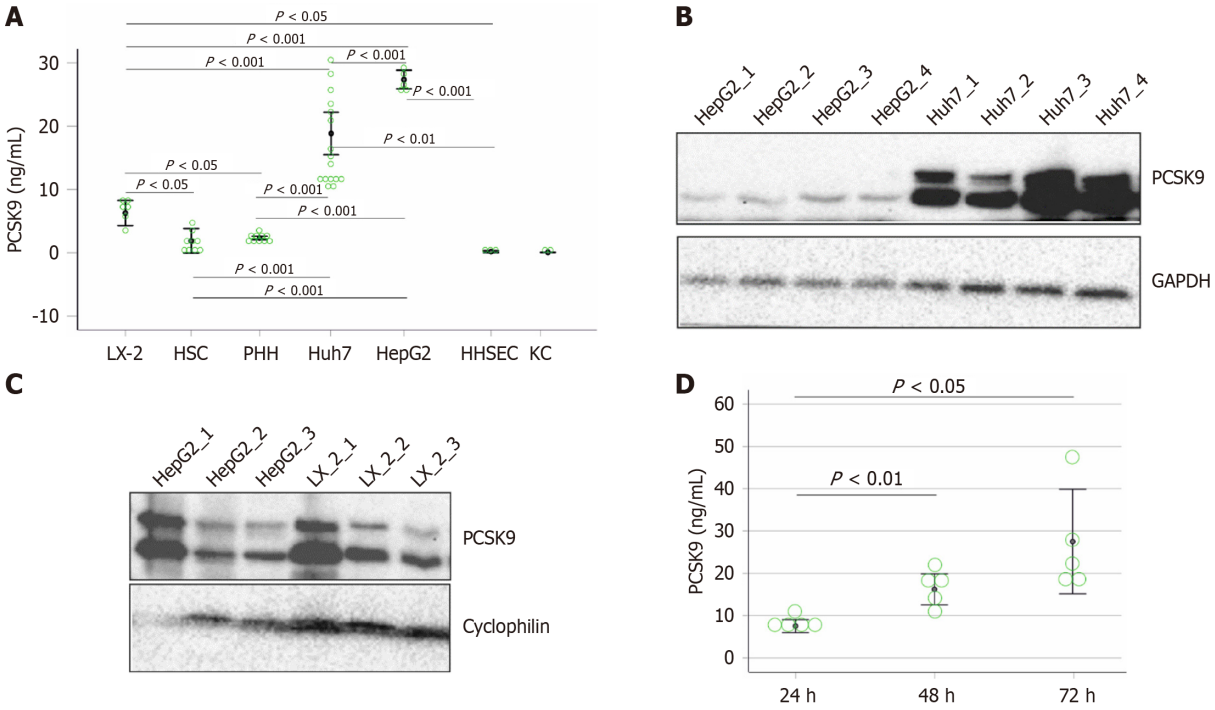

Figure 1 Proprotein convertase subtilisin/kexin type 9 in the supernatants of 10^6 liver cells cultivated for 24 hours, cellular levels of HepG2, Huh7, and LX-2 cells, and of LX-2 cells during culture. A: Proprotein convertase subtilisin/kexin type 9 (PCSK9) in the supernatant of LX-2 cells (6 different cultures), primary human hepatic stellate cells from 9 donors, primary human hepatocytes from 10 donors, Huh7 cells (16 different cultures), HepG2 cells (5 different cultures), human hepatic sinusoidal endothelial cells from 3 donors and primary human Kupffer cells from 2 donors; B: Immunoblot of PCSK9 protein in Huh7 and HepG2 cells, glyceraldehyde-3-phosphate dehydrogenase was used for normalization; C: Immunoblot of PCSK9 protein in LX-2 and HepG2 cells, cyclophilin A was used for normalization; D: PCSK9 protein in the supernatant of LX-2 cells cultivated for 24 hours, 48 hours, and 72 hours. PCSK9: Proprotein convertase subtilisin/kexin type 9; HSC: Hepatic stellate cell; PHH: Primary human hepatic; HHSEC: Human hepatic sinusoidal endothelial cell; KC: Kupffer cell; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

Effect of nuclear receptor agonists

Ligands of PPAR-γ, LXR, and FXR inhibit the activation of HSCs[22,24]. When used at concentrations of 1 μmol/L and 2 μmol/L, the FXR agonist GW4064 did not alter PCSK9 levels in the cell medium (Figure 3F). The LXR agonist T0901317 reduced PCSK9 in the cell medium at a concentration of 10 μmol/L but had no effect at 5 μmol/L (Figure 3G). Rosiglitazone, which activates PPAR-γ, had no effect at concentrations of 7.5 μmol/L and 15 μmol/L (Figure 3H).

DISCUSSION

This study demonstrated that PCSK9 is abundantly expressed in LX-2 cells. PCSK9 is downregulated in the cell and the cell medium when the cells are incubated with the fibrotic cytokine TGF-β. The LXR ligand, which exhibits antifibrotic properties, also reduces PCSK9 levels in the LX-2 cell medium. Therefore, PCSK9 levels in the cell medium of LX-2 cells are not associated with HSC activation.

Previous studies had suggested that hepatocytes were the main source of serum PCSK9[53]. This study demonstrated that HSCs secrete comparable levels of PCSK9 to PHHs. However, as hepatocytes make up the majority of cells in the liver[1], they are likely to be the most important contributors to blood PCSK9 levels. Whether HSCs also significantly contribute to circulating PCSK9 levels cannot be answered by our descriptive study. It is unlikely that Kupffer cells and

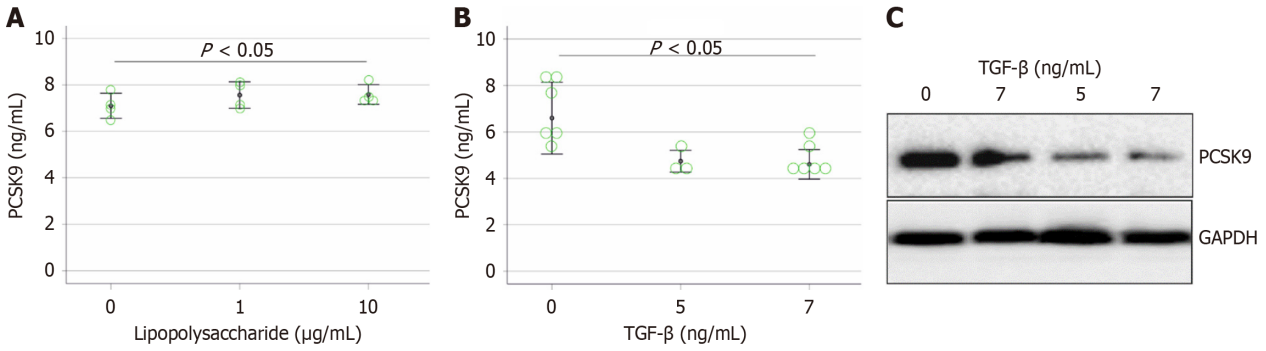

Figure 2 Effect of lipopolysaccharide and transforming growth factor beta on proprotein convertase subtilisin/kexin type 9 in the culture medium of LX-2 cells. A: Proprotein convertase subtilisin/kexin type 9 (PCSK9) in the LX-2 cell culture medium after 24 hours of cultivation with 0 μg/mL, 1 μg/mL, or 10 μg/mL lipopolysaccharide (4 independent experiments); B: PCSK9 in the LX-2 cell culture medium after 24 hours of cultivation with 0 ng/mL, 5 ng/mL, or 7 ng/mL transforming growth factor beta (TGF-β; 6 independent experiments for controls and 7 ng TGF-β, and 3 independent measures for 5 ng/mL); C: PCSK9 protein of LX-2 cells cultivated with 0 ng/mL, 5 ng/mL, or 7 ng/mL TGF-β for 24 hours. PCSK9: Proprotein convertase subtilisin/kexin type 9; TGF-β: Transforming growth factor beta; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

endothelial sinusoidal cells affect serum PCSK9 concentrations, given that they both have very low PCSK9 levels in their cell culture medium. Macrophages were shown to express cellular PCSK9 protein in one study[54], whereas another study did not detect it in these cells[55].

Interestingly, hepatocyte cell lines and LX-2 cells had higher PCSK9 levels than the respective primary cells. Esophageal squamous cell carcinoma cells exhibited higher PCSK9 expression than non-tumor esophageal tissues[56]. In hepatocellular carcinoma, both high and low expression of PCSK9 have been reported in tumors. Inhibiting PCSK9 in Huh7 and HepG2 cells reduced the proliferation of both cell lines, showing that PCSK9 contributes to tumor progression [57]. From our analysis, it is suggested that PCSK9 is increased in parenchymal liver tumor cells, and is also induced in the LX-2 cell line compared to primary HSCs. Unlike cell lines, primary hepatocytes and Kupffer cells do not proliferate during culture. Therefore, having a higher number of these cells in the study sample at the end of the experiment may also contribute to their increased PCSK9 levels. The doubling time is 2 days for HepG2 and Huh7 cells[58] and 3 days to 4 days for LX-2 cells[21]. Although we calculated PCSK9 levels relative to the number of cells plated, we did not determine the number of cells at the end of the study. Taking proliferation into account, therefore, PCSK9 levels in the culture medium of HepG2 and Huh7 cells are likely to be about 30% lower and 20% lower in LX-2 cells.

PCSK9 levels were higher in the cell culture medium of HepG2 cells than in Huh7 cells, but cellular expression was lower. This shows that cellular expression and secretion of PCSK9 are not necessarily correlated. It is suggested that increased PCSK9 release from HepG2 cells is associated with lower cellular levels and vice versa in Huh7 cells. This hypothesis needs to be evaluated in the future.

HSC and LX-2 cells secrete considerable levels of PCSK9, and this study analyzed the regulation of PCSK9 in the LX-2 cell line by LPS, cytokines, and the adipokines adiponectin, leptin, and chemerin known to regulate HSC activation. This study further investigated the effect of nuclear receptor agonists, all of which are described as exerting antifibrotic activities in HSCs[4,22,59]. Overall, the impact of these agents on PCSK9 levels in the cell culture medium was modest yet significant.

IL-6, which induces the acute-phase response[6], was shown to reduce PCSK9 levels. In rheumatoid arthritis synovial fibroblasts, IL-6 was found to lower PCSK9 enzymatic activity[60]. Knockdown of signal transducer and activator of transcription 3 (STAT3) induced PCSK9 in HepG2 cells. This is consistent with lower PCSK9 levels following IL-6 stimulation, which activates STAT3[6,31]. Thus, IL-6 downregulates PCSK9 in different cells including HSCs.

However, TNF, which is also an inflammatory cytokine[61], had no effect on the PCSK9 levels in LX-2 cells. In HepG2 cells, TNF increased PCSK9 levels[31] but lowered PCSK9 in rheumatoid arthritis synovial fibroblasts[60], demonstrating that the effect of TNF is cell-type specific.

LPS was the only agent to induce PCSK9 levels in the medium of LX-2 cells. However, this effect was modest, at less than 10% compared to the controls. LPS increased PCSK9 mRNA in the murine liver[32], which is principally concordant with our observation. This study did not perform cell type-specific analysis[32], and mRNA expression was not studied in the present investigation; therefore, it is difficult to compare these results. In humans, endotoxin infusion did not change plasma PCSK9 levels[62]. Notably, LPS was found to strongly reduce PCSK9 expression in human gut xenografts [63]. Overall, the regulation of PCSK9 by TNF and LPS is more complex than was initially suggested.

It is well documented that chronic inflammation lowers serum LDL levels[64], which is compatible with low PCSK9. However, the level of PCSK9 in the blood increases in cases of sepsis, whereas the level of LDL decreases[25,64,65]. First, this suggests that PCSK9 is not essential for maintaining LDL levels in these patients. Second, the factors contributing to high PCSK9 and low LDL levels in inflammation remain unclear.

Adipokines have emerged as essential proteins that target almost all of the body's organs[14,66-68]. Adiponectin protects against liver disease and fibrosis, whereas leptin promotes fibrosis[13,14]. Adiponectin receptor agonists were found to increase PCSK9 levels in HepG2 cells. In wild-type mice, this agonist increased PCSK9 levels, whereas in apolipoprotein E-deficient mice, which are prone to atherosclerosis, it lowered them[37]. However, in LX-2 cells, adiponectin did not regulate PCSK9 levels in the cell medium. These results suggest that the regulation of PCSK9 by

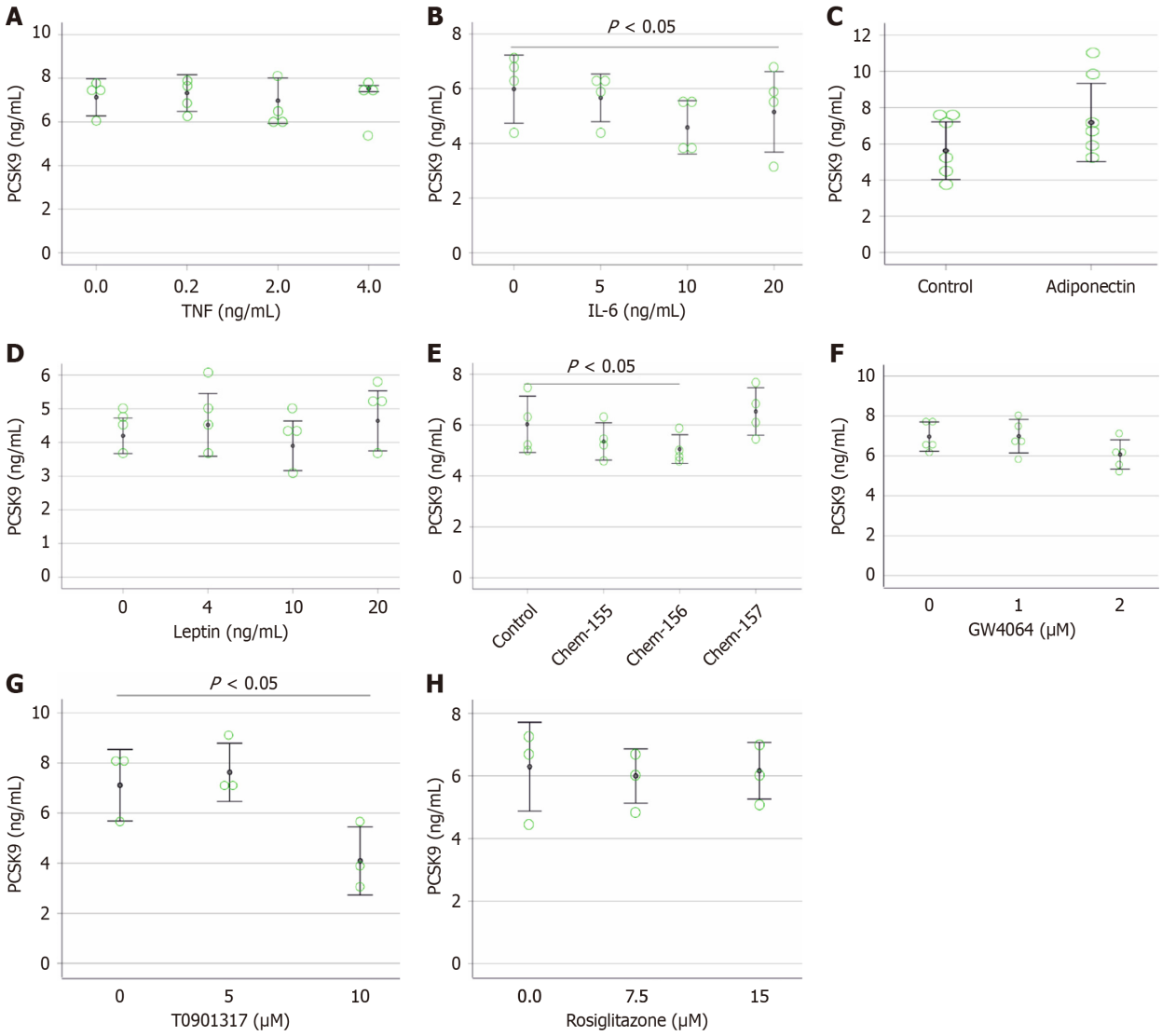

Figure 3 Regulation of proprotein convertase subtilisin/kexin type 9 levels in LX-2 cells by cytokines, adipokines, and nuclear receptor agonists. A and B: Proprotein convertase subtilisin/kexin type 9 (PCSK9) levels in LX-2 cells cultivated with tumor necrosis factor and interleukin-6. PCSK9 levels in the LX-2 cell supernatant after 24 hours of cultivation with 0 ng/mL, 0.2 ng/mL, 2 ng/mL, or 4 ng/mL tumor necrosis factor, 4 independent experiments (A); PCSK9 levels in the LX-2 cell supernatant after 24 hours of cultivation with 0 ng/mL, 5 ng/mL, 10 ng/mL, or 20 ng/mL interleukin-6, 4 independent experiments (B); C-E: PCSK9 levels during LX-2 cell cultivation with adipokines and chemerin isoform overexpression. PCSK9 levels in the LX-2 cell culture supernatant after 24 hours of cultivation with 0 μg/mL or 10 μg/mL adiponectin, 6 independent experiments (C); PCSK9 levels in the LX-2 cell culture supernatant after 24 hours of cultivation with 0 ng/mL, 4 ng/mL, 10 ng/mL, or 20 ng/mL leptin, 4 independent experiments (D); PCSK9 in the supernatant of LX-2 cells cultivated for 24 hours with overexpressed chemerin-155, chemerin-156, or chemerin-157 and in control transfected cells, 4 independent experiments (E); F-H: PCSK9 levels during LX-2 cell cultivation with nuclear receptor agonists. PCSK9 levels in the LX-2 cell culture supernatant after 24 hours of cultivation with 0 μmol/L, 1 μmol/L, and 2 μmol/L GW4064, 5 independent experiments (F); PCSK9 levels in the LX-2 cell culture supernatant after 24 hours of cultivation with 0 μmol/L, 5 μmol/L, and 10 μmol/L T0901317, 3 independent experiments (G); PCSK9 in the supernatant of LX-2 cells cultivated for 24 hours with 0 μmol/L, 7.5 μmol/L, and 15 μmol/L rosiglitazone, 3 independent experiments (H). PCSK9: Proprotein convertase subtilisin/kexin type 9; TNF: Tumor necrosis factor; IL: Interleukin; Chem: Chemerin.

adiponectin and its receptor agonists requires further study.

Leptin was also shown to increase PCSK9 levels in HepG2 cells, and STAT3 was involved in the upregulation of PCSK9 [34]. These studies used concentrations of leptin of 50 ng/mL and 100 ng/mL, which were higher than those used in the current analysis. When used at concentrations of up to 20 ng/mL, leptin did not modulate PCSK9 levels in LX-2 cells. Moreover, it was not analyzed whether leptin also increases PCSK9 in the cell medium of the HepG2 cells[34]. Currently it is unclear whether leptin has a role in PCSK9 secretion of hepatocytes.

Chemerin-156, an isoform of chemerin related to the activation of HSCs when overexpressed[17], has been shown to reduce PCSK9 levels of LX-2 cells. Chemerin was originally described as a chemotactic protein; meanwhile, various functions of chemerin, such as its role in glucose metabolism, hypertension, and carcinogenesis, have been identified[16]. The effect of chemerin on PCSK9 expression in liver cells has not been studied, to our knowledge.

The strongest effect on PCSK9 levels was exerted by TGF-β, which lowered PCSK9 in cell medium and cell lysate by approximately 30%. TGF-β is a well-described profibrotic protein that activates HSCs and stimulates the synthesis of the extracellular matrix[19]. TGF-β inhibits the transcription of cholesterol biosynthesis genes in hepatocytes[47], such as

sterol regulatory element-binding protein 2 (SREBP-2), which induces PCSK9 expression[25]. HSC activation is associated with cholesterol depletion and reduced expression of the LDL receptor, which is a target gene of SREBP-2[69], consistent with reduced SREBP-2 activity. Therefore, it is likely that the low levels of SREBP-2 in TGF-β-treated HSCs contribute to the reduction in cellular and soluble PCSK9 levels.

Ligands of the nuclear receptors FXR, LXR, and PPAR-γ can counteract HSC activation[22,24]. However, the LXR ligand also lowered PCSK9 levels in the cell medium. LXR activation was shown to increase cholesterol efflux, thereby reducing cellular cholesterol levels[70]. LXR agonists have also been shown to reduce LDL receptor protein levels on the cell surface[70,71]. One study found this effect to be independent of PCSK9, whereas a second analysis found it to be associated with higher PCSK9 levels[71,72]. In LX-2 cells, the LXR agonist reduced PCSK9, which is consistent with the PCSK9-independent lowering of LDL receptors in these cells.

Low cellular cholesterol levels and suppression of SREBP-2 are common to both LXR agonist-activated and TGF-β-activated HSCs[47,71,73]. This suggests that the reduced level of PCSK9 in the cell medium is due to the reduced activity of SREBP-2 in these cells. Loading HSCs with aggregated LDL increased PCSK9 levels in the cell supernatant, consistent with cholesterol-dependent regulation[30]. However, the depletion or overexpression of SREBP-2 affect PCSK9 levels either way, so it is difficult to prove whether this transcription factor is involved in the effects of TGF-β and LXR.

This study did not evaluate the signaling pathways underlying the regulation of PCSK9 levels by LPS, IL-6, TGF-b and chemerin-156. Both TGF-β and LPS activate mothers against decapentaplegic homolog 2 (SMAD2) in LX-2 cells[19,74], but have opposing effects on PCSK9 in these cells. IL-6 and TNF activate STAT3[6,75], but only IL-6 reduced the level of PCSK9 in the cell medium of LX-2 cells. The signaling pathways that regulate PCSK9 levels in LX-2 cells are currently unclear, as is the question of whether PCSK9 transcription, post-transcriptional processing, or secretion is affected by the factors studied herein. The scope of these studies is too extensive for this investigation.

The role of PCSK9 in HSCs has yet to be determined. It is unclear whether PCSK9 contributes to the cellular cholesterol levels of these cells. The serum PCSK9 levels of patients with liver cirrhosis are reduced[25], which is consistent with the downregulation of PCSK9 by TGF-β. However, liver synthesis capacity is low in cirrhosis[76] and may be the main reason for reduced PCSK9 levels in the circulation.

This study had several limitations. The cells were incubated for 24 hours, and the effects of incubation for longer or shorter time intervals were not tested. Furthermore, cellular PCSK9 protein levels are not necessarily correlated with secreted levels[38], and cellular PCSK9 proteins were mostly not analyzed. Finally, we used the LX-2 cell line in our study, which may behave differently from primary HSCs.

Future studies should analyze the levels of mRNA and PCSK9 protein (both cellular and secreted) in HSCs from different donors, using different concentrations of IL-6, TGF-b, chemerin-156, and LPS for varying incubation times. This could provide insights into the signaling pathways that regulate PCSK9 levels in HSCs. Future work should focus on clarifying the role of cellular and secreted PCSK9 in these cells. Immunohistochemistry may be used to determine the expression of PCSK9 in biopsies of patients with liver fibrosis of different etiologies.

CONCLUSION

This study is the first to compare the levels of PCSK9 in different liver cells and to demonstrate that HSCs secrete considerable amounts of PCSK9. It was shown that IL-6, TGF-β, chemerin-156, and LXR activation all reduce PCSK9 levels in the LX-2 cell culture medium, whereas LPS induces them. From these *in vitro* data, it can be concluded that secreted PCSK9 is reduced in activated HSCs, as most inflammatory agents, as well as TGF-β, lowered it levels.

ACKNOWLEDGEMENTS

The excellent support of Elena Underberg is greatly acknowledged.

FOOTNOTES

Author contributions: Bundschuh J, Neumann M, Zimny S, and Spirk M performed the study; Bundschuh J and Buechler C analyzed the data; Buechler C designed the research study and wrote the manuscript. All authors have read and approved the final manuscript.

Institutional review board statement: Experimental procedures were performed according to the guidelines of the charitable state-controlled foundation Human Tissue and Cell Research, with the written informed patient's consent approved by the local ethical committee of the University Hospital of Regensburg (Approval No. 12-101-0048).

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The dataset supporting the conclusions of this article is available from the corresponding author.

Country of origin: Germany

ORCID number: Christa Buechler 0000-0002-5635-3994.

S-Editor: Zuo Q
L-Editor: A
P-Editor: Xu J

REFERENCES

1 **Ishibashi H**, Nakamura M, Komori A, Migita K, Shimoda S. Liver architecture, cell function, and disease. *Semin Immunopathol* 2009; **31**: 399-409 [*RCA*] [PMID: 19468732 DOI: 10.1007/s00281-009-0155-6] [FullText]
2 **Moriwaki H**, Blaner WS, Piantedosi R, Goodman DS. Effects of dietary retinoid and triglyceride on the lipid composition of rat liver stellate cells and stellate cell lipid droplets. *J Lipid Res* 1988; **29**: 1523-1534 [*RCA*] [PMID: 3241127] [FullText]
3 **Yamada M**, Blaner WS, Soprano DR, Dixon JL, Kjeldbye HM, Goodman DS. Biochemical characteristics of isolated rat liver stellate cells. *Hepatology* 1987; **7**: 1224-1229 [*RCA*] [PMID: 2824313 DOI: 10.1002/hep.1840070609] [FullText]
4 **Kamm DR**, McCommis KS. Hepatic stellate cells in physiology and pathology. *J Physiol* 2022; **600**: 1825-1837 [*RCA*] [PMID: 35307840 DOI: 10.1113/JP281061] [FullText]
5 **Seki E**, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, Schwabe RF. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 2007; **13**: 1324-1332 [*RCA*] [PMID: 17952090 DOI: 10.1038/nm1663] [FullText]
6 **Schmidt-Arras D**, Rose-John S. IL-6 pathway in the liver: From physiopathology to therapy. *J Hepatol* 2016; **64**: 1403-1415 [*RCA*] [PMID: 26867490 DOI: 10.1016/j.jhep.2016.02.004] [FullText]
7 **Wang MJ**, Zhang HL, Chen F, Guo XJ, Liu QG, Hou J. The double-edged effects of IL-6 in liver regeneration, aging, inflammation, and diseases. *Exp Hematol Oncol* 2024; **13**: 62 [*RCA*] [PMID: 38890694 DOI: 10.1186/s40164-024-00527-1] [FullText] [Full Text(PDF)]
8 **Gandhi CR**. Hepatic stellate cell activation and pro-fibrogenic signals. *J Hepatol* 2017; **67**: 1104-1105 [*RCA*] [PMID: 28939135 DOI: 10.1016/j.jhep.2017.06.001] [FullText]
9 **Xiang DM**, Sun W, Ning BF, Zhou TF, Li XF, Zhong W, Cheng Z, Xia MY, Wang X, Deng X, Wang W, Li HY, Cui XL, Li SC, Wu B, Xie WF, Wang HY, Ding J. The HLF/IL-6/STAT3 feedforward circuit drives hepatic stellate cell activation to promote liver fibrosis. *Gut* 2018; **67**: 1704-1715 [*RCA*] [PMID: 28754776 DOI: 10.1136/gutjnl-2016-313392] [FullText]
10 **Elsabaawy M**. Liver at crossroads: unraveling the links between obesity, chronic liver diseases, and the mysterious obesity paradox. *Clin Exp Med* 2024; **24**: 240 [*RCA*] [PMID: 39402270 DOI: 10.1007/s10238-024-01493-y] [FullText] [Full Text(PDF)]
11 **Zakhari S**. Bermuda Triangle for the liver: alcohol, obesity, and viral hepatitis. *J Gastroenterol Hepatol* 2013; **28** Suppl 1: 18-25 [*RCA*] [PMID: 23855291 DOI: 10.1111/jgh.12207] [FullText]
12 **Fontana L**, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes* 2007; **56**: 1010-1013 [*RCA*] [PMID: 17287468 DOI: 10.2337/db06-1656] [FullText]
13 **Adolph TE**, Grander C, Grabherr F, Tilg H. Adipokines and Non-Alcoholic Fatty Liver Disease: Multiple Interactions. *Int J Mol Sci* 2017; **18**: 1649 [*RCA*] [PMID: 28758929 DOI: 10.3390/ijms18081649] [FullText] [Full Text(PDF)]
14 **Buechler C**, Haberl EM, Rein-Fischboeck L, Aslanidis C. Adipokines in Liver Cirrhosis. *Int J Mol Sci* 2017; **18**: 1392 [*RCA*] [PMID: 28661458 DOI: 10.3390/ijms18071392] [FullText] [Full Text(PDF)]
15 **Park PH**, Sanz-Garcia C, Nagy LE. Adiponectin as an anti-fibrotic and anti-inflammatory adipokine in the liver. *Curr Pathobiol Rep* 2015; **3**: 243-252 [*RCA*] [PMID: 26858914 DOI: 10.1007/s40139-015-0094-y] [FullText]
16 **Buechler C**, Feder S, Haberl EM, Aslanidis C. Chemerin Isoforms and Activity in Obesity. *Int J Mol Sci* 2019; **20**: 1128 [*RCA*] [PMID: 30841637 DOI: 10.3390/ijms20051128] [FullText] [Full Text(PDF)]
17 **Spirk M**, Zimny S, Neumann M, McMullen N, Sinal CJ, Buechler C. Chemerin-156 is the Active Isoform in Human Hepatic Stellate Cells. *Int J Mol Sci* 2020; **21**: 7555 [*RCA*] [PMID: 33066326 DOI: 10.3390/ijms21207555] [FullText] [Full Text(PDF)]
18 **Lodyga M**, Hinz B. TGF-β1 - A truly transforming growth factor in fibrosis and immunity. *Semin Cell Dev Biol* 2020; **101**: 123-139 [*RCA*] [PMID: 31879265 DOI: 10.1016/j.semcdb.2019.12.010] [FullText]
19 **Xu F**, Liu C, Zhou D, Zhang L. TGF-β/SMAD Pathway and Its Regulation in Hepatic Fibrosis. *J Histochem Cytochem* 2016; **64**: 157-167 [*RCA*] [PMID: 26747705 DOI: 10.1369/0022155415627681] [FullText]
20 **Zimny S**, Pohl R, Rein-Fischboeck L, Haberl EM, Krautbauer S, Weiss TS, Buechler C. Chemokine (CC-motif) receptor-like 2 mRNA is expressed in hepatic stellate cells and is positively associated with characteristics of non-alcoholic steatohepatitis in mice and men. *Exp Mol Pathol* 2017; **103**: 1-8 [*RCA*] [PMID: 28600126 DOI: 10.1016/j.yexmp.2017.06.001] [FullText]
21 **Xu L**, Hui AY, Albanis E, Arthur MJ, O'Byrne SM, Blaner WS, Mukherjee P, Friedman SL, Eng FJ. Human hepatic stellate cell lines, LX-1 and LX-2: new tools for analysis of hepatic fibrosis. *Gut* 2005; **54**: 142-151 [*RCA*] [PMID: 15591520 DOI: 10.1136/gut.2004.042127] [Full Text]
22 **Yan M**, Cui Y, Xiang Q. Metabolism of hepatic stellate cells in chronic liver diseases: emerging molecular and therapeutic interventions. *Theranostics* 2025; **15**: 1715-1740 [*RCA*] [PMID: 39897543 DOI: 10.7150/thno.106597] [FullText] [Full Text(PDF)]
23 **Zhang S**, Wang J, Liu Q, Harnish DC. Farnesoid X receptor agonist WAY-362450 attenuates liver inflammation and fibrosis in murine model of non-alcoholic steatohepatitis. *J Hepatol* 2009; **51**: 380-388 [*RCA*] [PMID: 19501927 DOI: 10.1016/j.jhep.2009.03.025] [FullText]
24 **Miyahara T**, Schrum L, Rippe R, Xiong S, Yee HF Jr, Motomura K, Anania FA, Willson TM, Tsukamoto H. Peroxisome proliferator-activated receptors and hepatic stellate cell activation. *J Biol Chem* 2000; **275**: 35715-35722 [*RCA*] [PMID: 10969082 DOI: 10.1074/jbc.M006577200] [FullText]
25 **Grewal T**, Buechler C. Emerging Insights on the Diverse Roles of Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) in Chronic Liver

Diseases: Cholesterol Metabolism and Beyond. *Int J Mol Sci* 2022; **23**: 1070 [*RCA*] [PMID: 35162992 DOI: 10.3390/ijms23031070] [FullText] [Full Text(PDF)]

26 **Han L**, Wu L, Yin Q, Li L, Zheng X, Du S, Huang X, Bai L, Wang Y, Bian Y. A promising therapy for fatty liver disease: PCSK9 inhibitors. *Phytomedicine* 2024; **128**: 155505 [*RCA*] [PMID: 38547616 DOI: 10.1016/j.phymed.2024.155505] [FullText]

27 **Ruscica M**, Tokgözoğlu L, Corsini A, Sirtori CR. PCSK9 inhibition and inflammation: A narrative review. *Atherosclerosis* 2019; **288**: 146-155 [*RCA*] [PMID: 31404822 DOI: 10.1016/j.atherosclerosis.2019.07.015] [FullText]

28 **Bao H**, Wang X, Zhou H, Zhou W, Liao F, Wei F, Yang S, Luo Z, Li W. PCSK9 regulates myofibroblast transformation through the JAK2/STAT3 pathway to regulate fibrosis after myocardial infarction. *Biochem Pharmacol* 2024; **220**: 115996 [*RCA*] [PMID: 38154546 DOI: 10.1016/j.bcp.2023.115996] [FullText]

29 **Chung CC**, Kao YH, Chen YC, Lin YK, Higa S, Hsu KC, Chen YJ. PCSK9 Enhances Cardiac Fibrogenesis *via* the Activation of Toll-like Receptor and NLRP3 Inflammasome Signaling. *Int J Mol Sci* 2025; **26**: 1921 [*RCA*] [PMID: 40076547 DOI: 10.3390/ijms26051921] [Full Text] [Full Text(PDF)]

30 **Luquero A**, Vilahur G, Casani L, Badimon L, Borrell-Pages M. Differential cholesterol uptake in liver cells: A role for PCSK9. *FASEB J* 2022; **36**: e22291 [*RCA*] [PMID: 35344222 DOI: 10.1096/fj.202101660RR] [FullText]

31 **Ruscica M**, Ricci C, Macchi C, Magni P, Cristofani R, Liu J, Corsini A, Ferri N. Suppressor of Cytokine Signaling-3 (SOCS-3) Induces Proprotein Convertase Subtilisin Kexin Type 9 (PCSK9) Expression in Hepatic HepG2 Cell Line. *J Biol Chem* 2016; **291**: 3508-3519 [*RCA*] [PMID: 26668321 DOI: 10.1074/jbc.M115.664706] [FullText]

32 **Feingold KR**, Moser AH, Shigenaga JK, Patzek SM, Grunfeld C. Inflammation stimulates the expression of PCSK9. *Biochem Biophys Res Commun* 2008; **374**: 341-344 [*RCA*] [PMID: 18638454 DOI: 10.1016/j.bbrc.2008.07.023] [FullText] [Full Text(PDF)]

33 **Du Y**, Li S, Cui CJ, Zhang Y, Yang SH, Li JJ. Leptin decreases the expression of low-density lipoprotein receptor *via* PCSK9 pathway: linking dyslipidemia with obesity. *J Transl Med* 2016; **14**: 276 [*RCA*] [PMID: 27663646 DOI: 10.1186/s12967-016-1032-4] [FullText] [Full Text (PDF)]

34 **Macchi C**, Greco MF, Botta M, Sperandeo P, Dongiovanni P, Valenti L, Cicero AFG, Borghi C, Lupo MG, Romeo S, Corsini A, Magni P, Ferri N, Ruscica M. Leptin, Resistin, and Proprotein Convertase Subtilisin/Kexin Type 9: The Role of STAT3. *Am J Pathol* 2020; **190**: 2226-2236 [*RCA*] [PMID: 32798443 DOI: 10.1016/j.ajpath.2020.07.016] [FullText]

35 **Ding Z**, Wang X, Liu S, Zhou S, Kore RA, Mu S, Deng X, Fan Y, Mehta JL. NLRP3 inflammasome *via* IL-1β regulates PCSK9 secretion. *Theranostics* 2020; **10**: 7100-7110 [*RCA*] [PMID: 32641981 DOI: 10.7150/thno.45939] [FullText] [Full Text(PDF)]

36 **Bhat IA**, Kabeer SW, Reza MI, Mir RH, Dar MO. AdipoRon: A Novel Insulin Sensitizer in Various Complications and the Underlying Mechanisms: A Review. *Curr Mol Pharmacol* 2020; **13**: 94-107 [*RCA*] [PMID: 31642417 DOI: 10.2174/1874467212666191022102800] [Full Text]

37 **Sun L**, Yang X, Li Q, Zeng P, Liu Y, Liu L, Chen Y, Yu M, Ma C, Li X, Li Y, Zhang R, Zhu Y, Miao QR, Han J, Duan Y. Activation of Adiponectin Receptor Regulates Proprotein Convertase Subtilisin/Kexin Type 9 Expression and Inhibits Lesions in ApoE-Deficient Mice. *Arterioscler Thromb Vasc Biol* 2017; **37**: 1290-1300 [*RCA*] [PMID: 28546220 DOI: 10.1161/ATVBAHA.117.309630] [FullText]

38 **Xia XD**, Peng ZS, Gu HM, Wang M, Wang GQ, Zhang DW. Regulation of PCSK9 Expression and Function: Mechanisms and Therapeutic Implications. *Front Cardiovasc Med* 2021; **8**: 764038 [*RCA*] [PMID: 34782856 DOI: 10.3389/fcvm.2021.764038] [FullText] [Full Text(PDF)]

39 **Damm G**, Pfeiffer E, Burkhardt B, Vermehren J, Nüssler AK, Weiss TS. Human parenchymal and non-parenchymal liver cell isolation, culture and characterization. *Hepatol Int* 2013; **7**: 951-958 [*RCA*] [PMID: 26202025 DOI: 10.1007/s12072-013-9475-7] [FullText]

40 **Jeschke MG**, Klein D, Thasler WE, Bolder U, Schlitt HJ, Jauch KW, Weiss TS. Insulin decreases inflammatory signal transcription factor expression in primary human liver cells after LPS challenge. *Mol Med* 2008; **14**: 11-19 [*RCA*] [PMID: 18037968 DOI: 10.2119/2007-00062.Jeschke] [FullText]

41 **Chen M**, Liu J, Yang W, Ling W. Lipopolysaccharide mediates hepatic stellate cell activation by regulating autophagy and retinoic acid signaling. *Autophagy* 2017; **13**: 1813-1827 [*RCA*] [PMID: 29160747 DOI: 10.1080/15548627.2017.1356550] [FullText]

42 **Zhao S**, Jiang J, Jing Y, Liu W, Yang X, Hou X, Gao L, Wei L. The concentration of tumor necrosis factor-α determines its protective or damaging effect on liver injury by regulating Yap activity. *Cell Death Dis* 2020; **11**: 70 [*RCA*] [PMID: 31988281 DOI: 10.1038/s41419-020-2264-z] [FullText] [Full Text(PDF)]

43 **Wang S**, Link F, Han M, Chaudhary R, Asimakopoulos A, Liebe R, Yao Y, Hammad S, Dropmann A, Krizanac M, Rubie C, Feiner LK, Glanemann M, Ebert MPA, Weiskirchen R, Henis YI, Ehrlich M, Dooley S. The Interplay of TGF-β1 and Cholesterol Orchestrating Hepatocyte Cell Fate, EMT, and Signals for HSC Activation. *Cell Mol Gastroenterol Hepatol* 2024; **17**: 567-587 [*RCA*] [PMID: 38154598 DOI: 10.1016/j.jcmgh.2023.12.012] [FullText]

44 **Mourtzikou A**, Alepaki M, Stamouli M, Pouliakis A, Skliris A, Karakitsos P. Evaluation of serum levels of IL-6, TNF-α, IL-10, IL-2 and IL-4 in patients with chronic hepatitis. *Inmunología* 2014; **33**: 41-50 [*RCA*] [DOI: 10.1016/j.inmuno.2014.01.001] [FullText]

45 **Kohla MAS**, Attia A, Darwesh N, Obada M, Taha H, Youssef MF. Association of serum levels of transforming growth factor b1 with disease severity in patients with hepatocellular carcinoma. *Hepatoma Res* 2017; **3**: 294-301 [*RCA*] [DOI: 10.20517/2394-5079.2017.40] [FullText]

46 **Solhi R**, Lotfi AS, Lotfinia M, Farzaneh Z, Piryaei A, Najimi M, Vosough M. Hepatic stellate cell activation by TGFβ induces hedgehog signaling and endoplasmic reticulum stress simultaneously. *Toxicol In Vitro* 2022; **80**: 105315 [*RCA*] [PMID: 35051607 DOI: 10.1016/j.tiv.2022.105315] [FullText]

47 **Cheng J**, Luo Y, Li Y, Zhang F, Zhang X, Zhou X, Ji L. Sex- and body mass index-specific reference intervals for serum leptin: a population based study in China. *Nutr Metab (Lond)* 2022; **19**: 54 [*RCA*] [PMID: 35941672 DOI: 10.1186/s12986-022-00689-x] [FullText] [Full Text (PDF)]

48 **Karmiris K**, Koutroubakis IE, Xidakis C, Polychronaki M, Voudouri T, Kouroumalis EA. Circulating levels of leptin, adiponectin, resistin, and ghrelin in inflammatory bowel disease. *Inflamm Bowel Dis* 2006; **12**: 100-105 [*RCA*] [PMID: 16432373 DOI: 10.1097/01.MIB.0000200345.38837.46] [FullText]

49 **Beaven SW**, Wroblewski K, Wang J, Hong C, Bensinger S, Tsukamoto H, Tontonoz P. Liver X receptor signaling is a determinant of stellate cell activation and susceptibility to fibrotic liver disease. *Gastroenterology* 2011; **140**: 1052-1062 [*RCA*] [PMID: 21134374 DOI: 10.1053/j.gastro.2010.11.053] [FullText] [Full Text(PDF)]

50 **Kim JK**, Cho IJ, Kim EO, Lee DG, Jung DH, Ki SH, Ku SK, Kim SC. Hemistepsin A inhibits T0901317-induced lipogenesis in the liver. *BMB Rep* 2021; **54**: 106-111 [*RCA*] [PMID: 32843130 DOI: 10.5483/BMBRep.2021.54.2.111] [FullText] [Full Text(PDF)]

51 **Li J**, Kuruba R, Wilson A, Gao X, Zhang Y, Li S. Inhibition of endothelin-1-mediated contraction of hepatic stellate cells by FXR ligand.

PLoS One 2010; **5**: e13955 [*RCA*] [PMID: 21085652 DOI: 10.1371/journal.pone.0013955] [FullText] [Full Text(PDF)]

52 **Schneider CA**, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 2012; **9**: 671-675 [*RCA*] [PMID: 22930834 DOI: 10.1038/nmeth.2089] [FullText]

53 **Shapiro MD**, Tavori H, Fazio S. PCSK9: From Basic Science Discoveries to Clinical Trials. *Circ Res* 2018; **122**: 1420-1438 [*RCA*] [PMID: 29748367 DOI: 10.1161/CIRCRESAHA.118.311227] [FullText]

54 **Badimon L**, Luquero A, Crespo J, Peña E, Borrell-Pages M. PCSK9 and LRP5 in macrophage lipid internalization and inflammation. *Cardiovasc Res* 2021; **117**: 2054-2068 [*RCA*] [PMID: 32991689 DOI: 10.1093/cvr/cvaa254] [FullText]

55 **Jaén RI**, Povo-Retana A, Rosales-Mendoza C, Capillas-Herrero P, Sánchez-García S, Martín-Sanz P, Mojena M, Prieto P, Boscá L. Functional Crosstalk between PCSK9 Internalization and Pro-Inflammatory Activation in Human Macrophages: Role of Reactive Oxygen Species Release. *Int J Mol Sci* 2022; **23**: 9114 [*RCA*] [PMID: 36012389 DOI: 10.3390/ijms23169114] [FullText] [Full Text(PDF)]

56 **Wang H**, Guo Q, Wang M, Liu C, Tian Z. PCSK9 promotes tumor cell proliferation and migration by facilitating CCL25 secretion in esophageal squamous cell carcinoma. *Oncol Lett* 2023; **26**: 500 [*RCA*] [PMID: 37854863 DOI: 10.3892/ol.2023.14086] [FullText] [Full Text (PDF)]

57 **Alannan M**, Seidah NG, Merched AJ. PCSK9 in Liver Cancers at the Crossroads between Lipid Metabolism and Immunity. *Cells* 2022; **11**: 4132 [*RCA*] [PMID: 36552895 DOI: 10.3390/cells11244132] [FullText]

58 **Louisa M**, Suyatna FD, Wanandi SI, Asih PB, Syafruddin D. Differential expression of several drug transporter genes in HepG2 and Huh-7 cell lines. *Adv Biomed Res* 2016; **5**: 104 [*RCA*] [PMID: 27376043 DOI: 10.4103/2277-9175.183664] [FullText] [Full Text(PDF)]

59 **Hou W**, Syn WK. Role of Metabolism in Hepatic Stellate Cell Activation and Fibrogenesis. *Front Cell Dev Biol* 2018; **6**: 150 [*RCA*] [PMID: 30483502 DOI: 10.3389/fcell.2018.00150] [FullText] [Full Text(PDF)]

60 **Shanta MA**, Shaikh FS, Madarampalli B, Ahmed S. Elucidating the Expression and Role of Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) in Human Rheumatoid Arthritis Synovial Fibroblasts and a Rat Adjuvant-induced Arthritis Model [abstract]. *Arthritis Rheumatol* 2022; **74**: (suppl 9)

61 **Ham B**, Fernandez MC, D'Costa Z, Brodt P. The diverse roles of the TNF axis in cancer progression and metastasis. *Trends Cancer Res* 2016; **11**: 1-27 [*RCA*] [PMID: 27928197] [FullText]

62 **Heinzl MW**, Resl M, Klammer C, Egger M, Dieplinger B, Clodi M. Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Is Not Induced in Artificial Human Inflammation and Is Not Correlated with Inflammatory Response. *Infect Immun* 2020; **88**: e00842-e00819 [*RCA*] [PMID: 31843964 DOI: 10.1128/IAI.00842-19] [FullText] [Full Text(PDF)]

63 **Nissim-Eliraz E**, Nir E, Marsiano N, Yagel S, Shpigel NY. NF-kappa-B activation unveils the presence of inflammatory hotspots in human gut xenografts. *PLoS One* 2021; **16**: e0243010 [*RCA*] [PMID: 33939711 DOI: 10.1371/journal.pone.0243010] [FullText] [Full Text(PDF)]

64 **Hofmaenner DA**, Kleyman A, Press A, Bauer M, Singer M. The Many Roles of Cholesterol in Sepsis: A Review. *Am J Respir Crit Care Med* 2022; **205**: 388-396 [*RCA*] [PMID: 34715007 DOI: 10.1164/rccm.202105-1197TR] [FullText]

65 **Boyd JH**, Fjell CD, Russell JA, Sirounis D, Cirstea MS, Walley KR. Increased Plasma PCSK9 Levels Are Associated with Reduced Endotoxin Clearance and the Development of Acute Organ Failures during Sepsis. *J Innate Immun* 2016; **8**: 211-220 [*RCA*] [PMID: 26756586 DOI: 10.1159/000442976] [FullText]

66 **Booth A**, Magnuson A, Fouts J, Foster M. Adipose tissue, obesity and adipokines: role in cancer promotion. *Horm Mol Biol Clin Investig* 2015; **21**: 57-74 [*RCA*] [PMID: 25781552 DOI: 10.1515/hmbci-2014-0037] [FullText]

67 **Ding M**, Rzucidlo EM, Davey JC, Xie Y, Liu R, Jin Y, Stavola L, Martin KA. Adiponectin in the heart and vascular system. *Vitam Horm* 2012; **90**: 289-319 [*RCA*] [PMID: 23017720 DOI: 10.1016/B978-0-12-398313-8.00011-7] [FullText]

68 **Kamada Y**, Takehara T, Hayashi N. Adipocytokines and liver disease. *J Gastroenterol* 2008; **43**: 811-822 [*RCA*] [PMID: 19012034 DOI: 10.1007/s00535-008-2213-6] [FullText]

69 **Schinagl M**, Tomin T, Gindlhuber J, Honeder S, Pfleger R, Schittmayer M, Trauner M, Birner-Gruenberger R. Proteomic Changes of Activated Hepatic Stellate Cells. *Int J Mol Sci* 2021; **22**: 12782 [*RCA*] [PMID: 34884585 DOI: 10.3390/ijms222312782] [FullText] [Full Text (PDF)]

70 **Zhao C**, Dahlman-Wright K. Liver X receptor in cholesterol metabolism. *J Endocrinol* 2010; **204**: 233-240 [*RCA*] [PMID: 19837721 DOI: 10.1677/JOE-09-0271] [FullText]

71 **Zhang L**, Reue K, Fong LG, Young SG, Tontonoz P. Feedback regulation of cholesterol uptake by the LXR-IDOL-LDLR axis. *Arterioscler Thromb Vasc Biol* 2012; **32**: 2541-2546 [*RCA*] [PMID: 22936343 DOI: 10.1161/ATVBAHA.112.250571] [FullText]

72 **Huang Y**, Ning K, Li WW, Lin G, Hou CL, Wang MJ, Zhu YC. Hydrogen sulfide accumulates LDL receptor precursor *via* downregulating PCSK9 in HepG2 cells. *Am J Physiol Cell Physiol* 2020; **319**: C1082-C1096 [*RCA*] [PMID: 32938225 DOI: 10.1152/ajpcell.00244.2019] [Full Text]

73 **Wang B**, Tontonoz P. Liver X receptors in lipid signalling and membrane homeostasis. *Nat Rev Endocrinol* 2018; **14**: 452-463 [*RCA*] [PMID: 29904174 DOI: 10.1038/s41574-018-0037-x] [FullText]

74 **Kao YH**, Chen PH, Wu TY, Lin YC, Tsai MS, Lee PH, Tai TS, Chang HR, Sun CK. Lipopolysaccharides induce Smad2 phosphorylation through PI3K/Akt and MAPK cascades in HSC-T6 hepatic stellate cells. *Life Sci* 2017; **184**: 37-46 [*RCA*] [PMID: 28689803 DOI: 10.1016/j.lfs.2017.07.004] [FullText]

75 **Antonia RJ**, Karelehto E, Toriguchi K, Matli M, Warren RS, Pfeffer LM, Donner DB. STAT3 regulates inflammatory cytokine production downstream of TNFR1 by inducing expression of TNFAIP3/A20. *J Cell Mol Med* 2022; **26**: 4591-4601 [*RCA*] [PMID: 35841281 DOI: 10.1111/jcmm.17489] [FullText] [Full Text(PDF)]

76 **Sharma P**. Value of Liver Function Tests in Cirrhosis. *J Clin Exp Hepatol* 2022; **12**: 948-964 [*RCA*] [PMID: 35677506 DOI: 10.1016/j.jceh.2021.11.004] [FullText]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: office@baishideng.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com