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Curcumin Acts Synergistically with Metformin to Inhibit Autophagy and Promote Pyroptosis in Hepatic Stellate Cells

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Abstract: Background: Hepatic stellate cells play a critical role in liver fibrosis progression through activation and proliferation. Autophagy and pyroptosis have both been reported to play key roles in the activation of HSCs. Objectives: This study aimed to observe the effects of cooperation between curcumin and metformin regarding the regulation of autophagy and pyroptosis in HSCs. Methods: HSCs (line LX-2) and rat T-6 cells were treated with either curcumin or curcumin and metformin combined. The vitality and proliferation of HSCs were then measured, as were the expressions of microtubule-associated light chain 3 (LC3) and ubiquitin-binding protein 62 (P62) to investigate autophagy. The autophagosomes in the HSC cells were quantified using transmission electron microscopy. The pyroptosis-related proteins gasdermin D (GSDMD) and caspase-1 were also measured, as were the expression levels of the inflammatory cytokines lactate dehydrogenase (LDH) and interleukin-1β (IL-1β). Results: When combined with metformin, curcumin significantly reduced the activities of the LX-2 and T-6 cells and significantly induced pyroptosis, as observed by flow cytometry. Furthermore, the expressions of GSDMD and caspase-1 were higher in the curcumin+metformin treatment group than in the curcumin group, and more LDH and 1L-1β were released. Conclusion: When combined with metformin, curcumin can inhibit autophagy and proliferation activity in the HSCs, promote pyroptosis, and play a role in delaying the process of liver fibrosis.

Keywords: Curcumin; Metformin; Autophagy; Pyroptosis; Liver Fibrosis.

1. Background

Hepatic fibrosis is a pathological process in which excessive amounts of the extracellular matrix (ECM) are deposited, owing to the unbalanced synthesis and degradation of ECM. This can occur if the liver has been continuously injured by a liver damage factor (1). HSC activation and proliferation are key factors in the occurrence and development of hepatic fibrosis, the early stage of liver fibrosis (2).

Curcumin is a natural phenolic antioxidant that can be extracted from the ginger plant turmeric (Curcuma longa). Previous studies have confirmed that curcumin can suppress the expressions of various factors that promote fibrosis in liver tissue by inhibiting the formation of lipid peroxides. In this way, it can inhibit the autophagy and proliferation of HSCs, induce apoptosis, and effectively inhibit the development of liver fibrosis (3-5). Metformin is a commonly used biguanide antihyperglycemic drug. Studies have shown that it can improve liver steatosis, inflammation, and fibrosis (6).

Autophagy is an important pathophysiological process in which primary lysosomes process endogenous substrates in various eukaryotic cells. This process degrades certain long-lived proteins and damaged organelles in cells, which can fuse with intracellular lysosomes to form autophagic lysosomes (7, 8). These autophagic lysosomes maintain the intracellular metabolic balance by degrading the contents they encapsulate. In previous studies, primary HSCs and cell lines have often been observed to be accompanied by increased autophagy activity during activation and proliferation (9). Therefore, specific interventions regarding the autophagy activities of HSCs could constitute a new strategy and a new target for anti-fibrosis treatment (10).

Inflammatory cell death is known as pyroptosis, and it is mediated by caspase-1(11); recent studies have shown that caspase-1 is responsible for pyroptosis (12). A variety of intracellular and extracellular pathogenic factors promote the formation of inflammasomes in the body, through certain pathways. They activate the precursors caspase-1 and interleukin-1β, and these activated inflammatory factors can chemoattract inflammatory cells and lead to more inflammation after causing cell lysis, which ultimately leads to cell inflammatory death (i.e., pyroptosis) (13). Pyroptosis has been found to be related in the progression of many diseases.

2. Objectives

In this study, the effects of curcumin and metformin on the regulation of autophagy and pyrolysis were examined for human-derived LX-2 cells and T-6 cells from rats. The molecular mechanisms that occur when combining these two substances were explored in relation to the prevention and treatment of liver fibrosis; the findings could provide new research ideas for the early treatment of Liver cirrhosis.

3. Methods

3.1. Cell lines and Culture

Immortalized human line LX-2 HSCs and rat T-6 cells with simian vacuolation virus 40 (SV40) transfections (which exhibit features typical of activated HSCs) were gifted by Prof. Scott Friedman. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal

bovine serum at 37°C, in an incubator containing 5% carbon dioxide (CO_2).

3.2. Cell Grouping and Processing

The LX-2 and T-6 cell lines in the logarithmic growth phase were divided into four groups: blank control group (BC), which received no treatment; negative control group (NC), to which dimethyl sulfoxide (DMSO) diluent was added; curcumin group (Cur), to which 10 μM of curcumin diluted in DMSO was added; and curcumin and metformin group (Cur+Met), to which 10 μM of curcumin and 10 μM of metformin (both diluted in DMSO) were added. After the cell grouping treatment, the cultures were continued for 24h.

3.3. CCK-8 Assay

CCK-8 assays were used to determine cell viability. Cells from each group were seeded into a 96well plate (1 * 104 cells*well-1) in minimum essential medium (MEM) after the cell grouping treatment. The absorbance was measured using a Fluostar Galaxy microplate reader at 450 nm.

3.4. Transmission Electron Microscopy (TEM)

TEM was used to quantify the number of autophagosomes in the LX-2 and T-6 cells. 2% glutaraldehyde and 1% osmium tetroxide were used to immobilize the cells, followed by washing with 100 mM sodium phosphate buffer (pH7.2), dehydrating with ethanol, and embedding. With an H600 Transmission Electron Microscope, ultrathin sections (60-80 nm) were prepared using an ultramicrotome and stained with lead citrate-uranylacetate.

3.5. Western Blot Analysis

Proteins related to autophagy and pyrolysis were detected using Western blot analysis. After 24 h of cell grouping treatment, both groups were washed twice with phosphate-buffered saline (PBS), followed by addition of a radioimmunoprecipitation assay (RIPA) lysis solution. Afterward, samples were centrifuged at 4°C for 20 minutes at 12000 rpm for 30 minutes on ice. The supernatant was then taken for further analyses. After measuring the protein content, 20-μg protein samples were taken and electrophoresis was performed. In the following step, samples were transferred to a membrane and sealed with 5% blocked milk for 1 hour. Primary antibodies were then cultured overnight at 4°C. Secondary antibody was added after the membrane had been washed twice with tris-buffered saline containing Tween (TBST) and incubated for 1 hour at room temperature. Following that, TBST was applied three times to the membrane. A chemiluminescent agent was added, and the samples were then moved to a darkroom for exposure and to develop images. GAPDH was used as an internal control.

3.6. Flow Cytometry Analysis

To assess pyroptosis in the HSCs, pyroptosis/caspase-1 cell pyroptosis was analyzed in the HSCs. After the cell grouping treatment, the cells were digested and washed twice with PBS. Pyroptosis/caspase-1 was then analyzed using a pyroptosis/caspase-1 cell pyroptosis detection kit, following the manufacturer's instructions. A flow cytometry analysis was performed after staining the samples with propidium iodide (PI) in the dark for 5-15 minutes. The term pyroptotic cell refers to cells that are double positive for caspase-1 and PI.

3.7. Enzyme-linked Immunosorbent Assay (ELISA)

The expression levels of LDH and interleukin-1β (1L-1β) were detected via ELISA. To remove impurities and cell debris from the medium, each group was centrifuged for 20 minutes at 4°C at 1,000 g. Base on the manufacturer's instructions, IL-1b and IL-18 were detected by ELISA using the rat detection kit. A standard curve was generated using a standard product, to determine IL-1b and IL-18 concentrations in the supernatant using the microplate reader's absorbance at 450 nm.

3.8. Statistical Analysis

Two groups of data derived from at least three independent experiments were compared using either a student's t-test or a one-way ANOVA (more than two groups). A p-value of < 0.05 was considered statistically significant (*$p < 0.05$; **$p < 0.01$; *** $p < 0.001$). All graphs were created using the GraphPad Prism 7 program.

4. Results

4.1. Curcumin Cooperates with Metformin to Inhibit Proliferation of LX-2 and T-6 Cells

The CCK-8 assay of the LX-2 and T-6 cells revealed that the cell proliferation abilities of the Cur and Cur+Met groups both decreased compared to the Control group, showing an inhibitory effect on LX-2. The Cur+Met group exhibited the strongest synergistic inhibitory effect ($P < 0.05$; Fig. 1A, B).

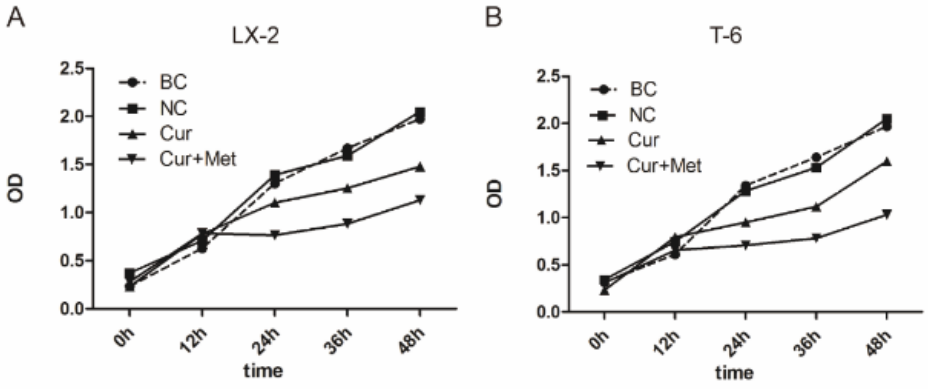

Figure 1. Exploration of cooperation between curcumin and metformin regarding inhibition of proliferation of LX-2 and T-6 cells. (A-B) Cell viability was determined by measuring the absorbance at 450 nm using LX-2 and T-6 cells. Optical density (OD) values are meant, plus a standard deviation (n = 3) to determine the number of viable cells

4.2. Curcumin and Metformin Synergistically Inhibited Autophagy

Western blot analysis revealed that for the LX-2 and T-6 cells, the expressions of the autophagy protein LC3II were lower in the Cur and Cur+Met groups than in the BC and NC groups (Fig. 2A, B); however, the expressions of p62 were slightly higher in the former two groups than in the latter ($P < 0.05$; Fig. 2C, D). The obtained TEM images showed that, compared with the BC and NC groups, the autophagosomes in the Cur+Met group were significantly reduced for the LX-2 and T-6 cells ($P < 0.05$), showing the formation of early autophagic lysosome vacuoles (Fig. 2E).

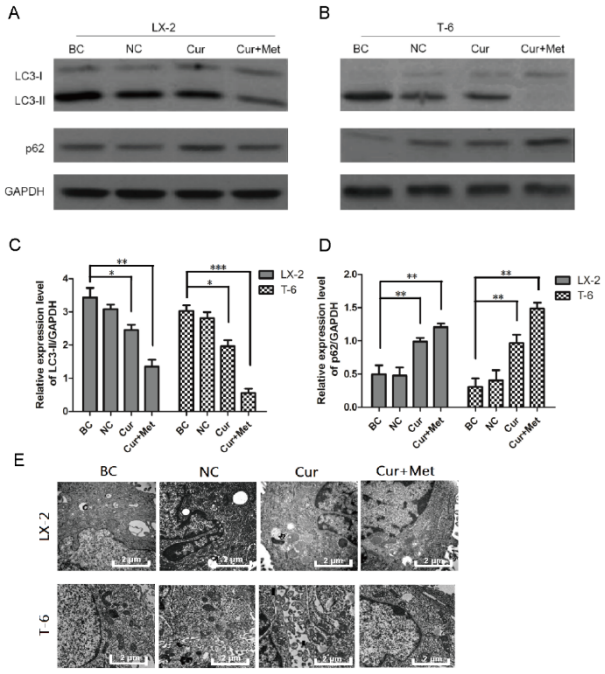

Figure 2. Quantification of densitometric data revealing synergistic effects of curcumin and metformin regarding autophagic flux. Th An intercellular control was GAPDH, and the autophagy markers LC3-II and p62 were measured using western blotting in LX-2 (A) and T-6 (B). (E)Representative images of autophagosomes in LX-2 and T-6 cells. Transmission electron microscopy (TEM) was used to visualize the ultrastructure of the cells (Scale bars = 2 μm). The results of three independent experiments are shown as means ± SD (**P* < 0.05, ***P* < 0.01, and ****P* < 0.001)

4.3. Curcumin and Metformin Synergistically Promote Pyroptosis of LX-2 and T-6 Cells

Gasdermin D (GSDMD) and caspase-1 are proteins closely related to pyroptosis. However, influences of curcumin and metformin in the pyroptosis of HSCs were investigated. Based on flow cytometry, the pyroptosis rates of LX-2 and T-6 cells were significantly higher in the Cur and Cur+Met groups than in the BC and NC groups (P 0.05; Fig. 3). The pyroptosis proteins analysis by western blot showed that among the two HSCs the expressions of caspase-1 and GSDMD in the Cur and Cur+Met groups gradually increased, with the increase in the Cur+Met group being the most obvious ($P < 0.05$; Fig. 3A-C). The ELISA test results showed that for the LX-2 and T-6 cells, the Cur and Cur+Met groups promoted the release of LDH and IL-1β, compared with the BC and NC groups. The expression levels of LDH (Fig. 4A, B) and IL-1β (Fig. 4C, D) increased in the Cur and Cur+Met groups, with the latter being significant ($P < 0.05$).

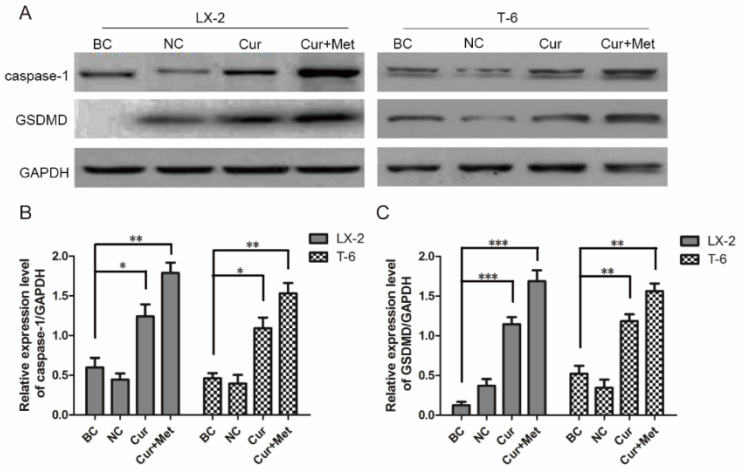

Figure 3. Synergistic effects of curcumin and metformin regarding pyroptosis of LX-2 and T-6 cells. Caspase-1 and gasdermin D (GSDMD) were measured by western blot analysis (A). Quantification of densitometric data of caspase-1 (B), and GSDMD (C) in cells. (*P < 0.05, **P < 0.01, and ***P < 0.001).

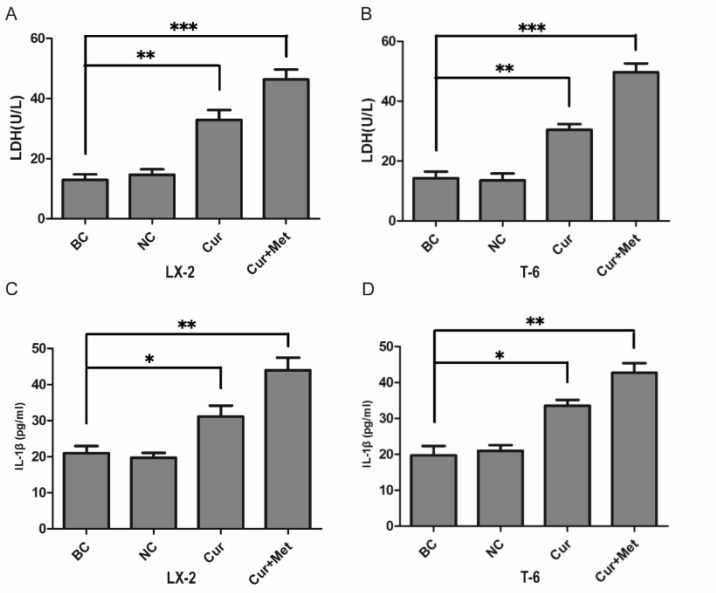

Figure 4. Synergistic effects of curcumin and metformin regarding the release of lactate dehydrogenase (LDH) and IL-1β. LDH and IL-β expressions were analyzed via ELISA. (*$P < 0.05$, and **$P < 0.01$ compare with the BC group).

5. Discussion

Liver fibrosis is a common pathophysiological process in both liver cirrhosis and primary liver cancer. It is an inherent repair response of the body to various chronic inflammations that lead to liver damage. HSCs have a resting type; the process by which they transform into their activated form is the central link in the occurrence of liver fibrosis (14). The timely and effective inhibition of the activation and proliferation of HSCs and the promotion of apoptosis, can block, reduce, or even reverse liver fibrosis (15). Therefore, further exploration is needed regarding the molecular mechanisms of HSC activation and liver fibrosis development. There is also a need to find new molecular regulatory targets regarding research into liver fibrosis (16).

Autophagy is an evolutionary conservative reaction that promotes liver homeostasis by removing misfolded proteins, damaged organelles, and lipid droplets to meet the needs of the body's own metabolic processes (17). The renewal of certain organelles also constitutes one of the manifestations of the process of programmed cell death. Studies have shown that the effects of autophagy are two-fold. For example, enhanced autophagy activity can protect hepatocytes from apoptosis but can also promote signal transduction during fibrosis formation (18).

Pyroptosis is a different type of cell death to natural cell death, apoptosis, or necrosis. It constitutes an immune defense response that initiates once the inflammatory body complex senses a pathogen signal, which the body initiates after sensing the pathogen's infection (19). As a result of caspase activation, the cell membrane is filled with micropores and vesicles, causing swelling and rupture, as well as the release of pro-inflammatory factors and cytokines (20). This process activates the body's natural immunity to trigger an inflammatory response, causing the osmotic disintegration of cells. The induction, development, and regulation of pyroptosis are closely related to the occurrences of many diseases, so it has become a research hotspot in recent years (21-23). Therefore, specific interventions regarding the autophagy and pyroptosis activities of HSCs would be conducive to the discovery of new strategies and new targets for clinical and basic anti-fibrosis research (24).

This study explored the effects of curcumin and metformin on the HSCs, from the perspectives of autophagy and pyroptosis. The results showed that treating LX-2 and T-6 cells with curcumin and metformin could inhibit their proliferation; curcumin and metformin could affect LX -2, while the inhibition of T-6 proliferation had a synergistic effect.

Related cell autophagy activity was determined to further explore the mechanism by which curcumin combined with metformin to inhibit the proliferation of HSCs. LC3 was the first autophagosome marker protein to be discovered; it participates in various stages of autophagosome formation to maturity, including LC3-I and LC3-II (25). When autophagy occurs, LC3-I is activated and then combines with phosphatidylethanolamine to transform into LC3-II, which promotes the formation and maturation of autophagosomes. As an autophagy receptor, p62 is specifically recruited to autophagosomes for degradation; it has been shown to be negatively correlated with the degree of autophagy (26, 27). Here, autophagosomes were observed via TEM and western blot analysis to detect autophagic proteins. The autophagosomes of the HSCs treated with curcumin combined with metformin were significantly reduced compared with the BC and NC groups. Furthermore, the expression of LC3II decreased while that of p62 increased, indicating that curcumin and metformin significantly inhibited the autophagy activity of HSCs.

GSDMD is a protein composed of 242 amino acids that is expressed on the surfaces of immune cells and small intestinal mucosal epithelial cells (28, 29). It is the common substrate of all inflammatory caspases and is the direct executor of pyroptosis. It mainly relies on caspase-1 to initiate the oligomerization of GSDMD-N, which in turn forms cell membrane pores (30); this causes cells to continue to expand until their envelope ruptures. Meanwhile, caspase-1 converts pro-inflammatory cytokine precursors (such as pro-IL-1) into mature cytokines (such as IL-1) (31). As the envelope ruptures, the cell contents are released, which recruits more

inflammatory cells to participate in the inflammatory response. Here, it was found that HSCs treated with curcumin combined with metformin exhibited higher pyroptosis rates, i.e., the formation of cell membrane pores increased, the expressions of the pyroptosis proteins GSDMD and caspase-1 increased, and LDH and 1L-1β were released. These results indicate that curcumin, when combined with metformin, can induce the activation of inflammasomes, promote the release of activated inflammatory cytokines, and promote the occurrence of pyroptosis (32, 33).

The main target of metformin is the liver; previous studies have found that it can improve liver steatosis, inflammation, and fibrosis. It can inhibit nuclear factor κB (NF-κB) activity and inflammation through AMPK-dependent and (or) independent pathways, respectively. The production of cytokines can inhibit inflammation and fibrosis (34). One of the mechanisms by which this occurs may involve increasing the liver's sensitivity to insulin, reducing hyperinsulinemia and insulin resistance, blocking continuous increases in triglycerides and free fatty acids, and thus delaying progress. Steatosis and liver cell ultrastructural mitochondrial lesions have been shown to decrease liver fibrogenic factors, such as smooth muscle actin and TGF-1, and to reduce the secretion of ECM by HSCs, thereby inhibiting liver fibrosis. This study found that the combination of curcumin and metformin enhanced the anti-fibrosis effect, further confirming that curcumin, when combined with metformin, has an anti-hepatic fibrosis effect.

6. Conclusion

In conclusion, curcumin, when combined with metformin, can inhibit the autophagy and proliferation activity of HSCs, promote the release of the inflammatory cytokines LDH and 1L-1β, induce inflammation, promote pyroptosis, and play a role in delaying the process of liver fibrosis.

Acknowledgments

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References

[1] Parola M, Pinzani M. Liver fibrosis: Pathophysiology, pathogenetic targets and clinical issues. Elsevier Ltd; 2019. p. 37-55.

[2] Higashi T, Friedman SL, Hoshida Y. Hepatic stellate cells as key target in liver fibrosis. Elsevier B.V.; 2017. p. 27-42.

[3] Liang WF, Gong YX, Li HF, Sun FL, Li WL, Chen DQ, et al. Curcumin Activates ROS Signaling to Promote Pyroptosis in Hepatocellular Carcinoma HepG2 Cells. In Vivo. 2021;35 (1):249-57.

[4] Kong D, Zhang Z, Chen L, Huang W, Zhang F, Wang L, et al. Curcumin blunts epithelial-mesenchymal transition of hepatocytes to alleviate hepatic fibrosis through regulating oxidative stress and autophagy. Redox Biol. 2020; 36:101600.

[5] Qin L, Qin J, Zhen X, Yang Q, Huang L. Curcumin protects against hepatic stellate cells activation and migration by inhibiting the CXCL12/CXCR4 biological axis in liver fibrosisA study in vitro and in vivo. Biomed Pharmacother. 2018; 101:599-607.

[6] Shankaraiah RC, Callegari E, Guerriero P, Rimessi A, Pinton P, Gramantieri L, et al. Metformin prevents liver tumourigenesis by attenuating fibrosis in a transgenic mouse model of hepatocellular carcinoma. Oncogene. 2019;38 (45): 7035-45.

[7] Wang L, Wang Y, Quan J. Exosomal miR-223 derived from natural killer cells inhibits hepatic stellate cell activation by suppressing autophagy. Mol Med. 2020;26(1):81.

[8] Liu XY, He YJ, Yang QH, Huang W, Liu ZH, Ye GR, et al. Induction of autophagy and apoptosis by miR-148a through the sonic hedgehog signaling pathway in hepatic stellate cells. Am J Cancer Res. 2015;5(9):2569-89.

[9] Polishchuk EV, Merolla A, Lichtmannegger J, Romano A, Indrieri A, Ilyechova EY, et al. Activation of Autophagy, Observed in Liver Tissues From Patients With Wilson Disease and From ATP7B-Deficient Animals, Protects Hepatocytes From Copper-Induced Apoptosis. Gastroenterology. 2019;156 (4): 1173-89 e5.

[10] Omar R, Yang J, Liu H, Davies NM, Gong Y. Hepatic Stellate Cells in Liver Fibrosis and siRNA-Based Therapy. Rev Physiol Biochem Pharmacol. 2016; 172:1-37.

[11] Shi J, Gao W, Shao F. Pyroptosis: Gasdermin-Mediated Programmed Necrotic Cell Death. Elsevier Ltd; 2017. p. 245-54.

[12] Liu W, Chen Y, Meng J, Wu M, Bi F, Chang C, et al. Ablation of caspase-1 protects against TBI-induced pyroptosis in vitro and in vivo. J Neuroinflammation. 2018;15(1):48.

[13] Wang Q, Wu J, Zeng Y, Chen K, Wang C, Yang S, et al. Pyroptosis: A pro-inflammatory type of cell death in cardiovascular disease. Clin Chim Acta. 2020; 510:62-72.

[14] Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. Nature Publishing Group; 2017. p. 397-411.

[15] Wallace MC, Friedman SL, Mann DA. Emerging and disease-specific mechanisms of hepatic stellate cell activation. Semin Liver Dis. 2015;35(2):107-18.

[16] Tsuchida T. [Mechanisms of hepatic stellate cell activation as a therapeutic target for the treatment of non-alcoholic steatohepatitis]. Nihon Yakurigaku Zasshi. 2019;154(4):203-9.

[17] Martinez-Lopez N, Singh R. Autophagy and Lipid Droplets in the Liver. Annu Rev Nutr. 2015; 35:215-37.

[18] Cai N, Zhao X, Jing Y, Sun K, Jiao S, Chen X, et al. Autophagy protects against palmitate-induced apoptosis in hepatocytes. Cell Biosci. 2014; 4:28.

[19] Ruan J, Wang S, Wang J. Mechanism and regulation of pyroptosis-mediated in cancer cell death. Chem Biol Interact. 2020; 323:109052.

[20] Shi J, Gao W, Shao F. Pyroptosis: Gasdermin-Mediated Programmed Necrotic Cell Death. Trends Biochem Sci. 2017;42(4):245-54.

[21] Sauler M, Bazan IS, Lee PJ. Cell Death in the Lung: The Apoptosis-Necroptosis Axis. Annu Rev Physiol. 2019; 81:375-402.

[22] Guo H, Xie M, Zhou C, Zheng M. The relevance of pyroptosis in the pathogenesis of liver diseases. Life Sci. 2019; 223:69-73.

[23] Zeng C, Wang R, Tan H. Role of Pyroptosis in Cardiovascular Diseases and its Therapeutic Implications. Int J Biol Sci. 2019;15(7):1345-57.

[24] Gaul S, Leszczynska A, Alegre F, Kaufmann B, Johnson CD, Adams LA, et al. Hepatocyte pyroptosis and release of

inflammasome particles induce stellate cell activation and liver fibrosis. J Hepatol. 2021;74(1):156-67.

[25] Xia F, Liu P, Li M. The regulatory factors and pathological roles of autophagy-related protein 4 in diverse diseases: Recent research advances. Med Res Rev. 2021;41(3):1644-75.

[26] Divya T, Sureshkumar A, Sudhandiran G. Autophagy induction by celastrol augments protection against bleomycin-induced experimental pulmonary fibrosis in rats: Role of adaptor protein p62/ SQSTM1. Pulm Pharmacol Ther. 2017; 45:47-61.

[27] Zhang XW, Zhou JC, Peng D, Hua F, Li K, Yu JJ, et al. Disrupting the TRIB3-SQSTM1 interaction reduces liver fibrosis by restoring autophagy and suppressing exosome-mediated HSC activation. Autophagy. 2020;16(5):782-96.

[28] Broz P, Pelegrin P, Shao F. The gasdermins, a protein family executing cell death and inflammation. Nat Rev Immunol. 2020;20(3):143-57.

[29] Qiu S, Liu J, Xing F. 'Hints' in the killer protein gasdermin D: unveiling the secrets of gasdermins driving cell death. Cell Death Differ. 2017;24(4):588-96.

[30] Van Opdenbosch N, Lamkanfi M. Caspases in Cell Death, Inflammation, and Disease. Immunity. 2019;50(6):1352-64.

[31] Schneider KS, Gross CJ, Dreier RF, Saller BS, Mishra R, Gorka O, et al. The Inflammasome Drives GSDMD-Independent Secondary Pyroptosis and IL-1 Release in the Absence of Caspase-1 Protease Activity. Cell Rep. 2017; 21 (13): 3846-59.

[32] Zhou Z, He H, Wang K, Shi X, Wang Y, Su Y, et al. Granzyme A from cytotoxic lymphocytes cleaves GSDMB to trigger pyroptosis in target cells. Science. 2020;368(6494).

[33] Liu X, Zhang Z, Ruan J, Pan Y, Magupalli VG, Wu H, et al. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. Nature. 2016;535(7610):153-8.

[34] Li Y, Chen Y. AMPK and Autophagy. Adv Exp Med Biol. 2019; 1206:85-108.