Proceedings of the 5th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/96/2025.22284

Different Cell Death Mechanisms of Non-alcoholic Fatty Liver Disease (NAFLD) and the Dawn of Targeted Therapy

Linli Xia

Guangdong Dublin College of Life Science and Technology (Sino-foreign cooperative education), South China Agricultural University, China
xialinli@foxmail.com

Abstract: According to recent epidemiological studies, the incidence of non-alcoholic fatty liver disease (NAFLD) has significantly increased globally, with estimates indicating that more than 25% of the world's population is impacted. Because of its close ties to obesity and metabolic dysfunction, NAFLD has become the most prevalent chronic liver disease in Western societies, even reaching epidemic proportions. The pathogenesis of NAFLD is closely associated with diverse modes of hepatocyte demise, which collectively drive inflammatory responses, fibrotic processes, and eventual hepatic functional impairment. The most well-researched of these mechanisms—apoptosis, necroptosis, pyroptosis, and ferroptosis—all have unique roles in the pathophysiology of NAFLD. Therefore, inhibiting cell death is a possible solution for NAFLD. This paper first discusses the pathophysiological mechanisms of NAFLD, and then discusses the mechanisms of four different existing cell death modes, particular cell death responses and molecular patterns in the onset of liver disease. The possibility of different cell death pathways as therapeutic targets is then discussed. In conclusion, this review synthesizes current knowledge on the contributions of distinct cell death mechanisms to NAFLD pathogenesis and explores their therapeutic potential by linking targeted treatment strategies to specific cell death pathways, thereby identifying novel opportunities for clinical intervention.

Keywords: Non-alcoholic fatty liver disease (NAFLD), cell death, apoptosis, necroptosis, ferroptosis

1. Introduction

Hepatic disease progression is modulated by multiple cell death modalities, including apoptosis, necrosis, and necroptosis, each initiating distinct signaling cascades that exacerbate pathological changes through divergent molecular pathways [1]. About 25% of the world's population suffers from non-alcoholic fatty liver disease (NAFLD), which is a major cause of chronic liver disease globally [2]. NAFLD represents a continuum of hepatic pathologies, evolving from uncomplicated steatosis to more severe manifestations such as steatohepatitis (NASH), fibrotic scarring, cirrhosis, and in advanced cases, hepatocellular carcinoma. The progression of NAFLD is driven by a complex interplay of metabolic, genetic, and environmental factors, with insulin resistance and lipotoxicity playing central roles [3]. Hepatocyte damage and death are caused by lipotoxicity, oxidative stress, and inflammation, all of which are brought on by a significant accumulation of lipids in the cells.

© 2025 The Authors. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

DOI: 10.54254/2753-8818/96/2025.22284

Cell death mechanisms are critical drivers of NAFLD progression. These pathways not only contribute to hepatocyte loss but also trigger inflammatory responses and activate hepatic stellate cells (HSCs), leading to fibrosis [4]. Apoptosis, a regulated form of cell death, is a hallmark of NAFLD, particularly in the transition from steatosis to NASH. Both pyroptosis and necroptosis are inflammatory cell death processes that worsen liver damage by releasing pro-inflammatory cytokines and damage-associated molecular patterns (DAMPs). New research identifies ferroptosis, a process for cell death reliant on iron buildup and lipid oxidative damage, as a key factor in the development of NAFLD [5].

Understanding the mechanisms underlying these cell death pathways is essential for developing targeted therapies. Current treatments for NAFLD focus on lifestyle modifications and pharmacological interventions to address metabolic dysfunction and reduce inflammation. However, emerging therapies targeting specific cell death pathways, such as caspase inhibitors, necroptosis inhibitors, and ferroptosis inhibitors, hold promise for improving clinical outcomes [6]. The cellular processes of apoptosis, necroptosis, pyroptosis, and ferroptosis in NAFLD are examined in this article along with their involvement in the development of the disease and possible treatment approaches aimed at attacking these pathways.

2. Mechanisms of NAFLD

NAFLD is hallmarked by excessive accumulation of lipid in hepatocytes, leading to lipotoxicity, oxidative stress, and inflammation. The "multiple-hit" hypothesis suggests that NAFLD progression involves a combination of genetic, metabolic, and environmental factors. Insulin resistance is a key driver, promoting lipolysis in adipose tissue and increasing free fatty acid (FFA) delivery to the liver. FFAs are esterified into triglycerides, which accumulate in hepatocytes, causing steatosis [7].

Lipotoxicity, caused by the accumulation of toxic lipid species like ceramides and diacylglycerols, leads to mitochondrial dysfunction and endoplasmic reticulum (ER) stress, causing oxidative stress and activating inflammatory pathways [8]. Inflammatory cytokines like TNF-α and IL-6 exacerbate hepatocyte injury and promote the activation of hepatic stellate cells, resulting in fibrosis [9].

The gut-liver axis plays a vital part in the pathophysiology of nonalcoholic fatty liver disease. Since dysbiosis increases intestinal permeability and permits bacterial products like lipopolysaccharide (LPS) to enter the portal circulation, stimulating Toll-like receptor 4 (TLR4) on Kupffer cells and causing liver inflammation and fibrosis [10].

3. Cell Death Mechanisms of NAFLD

Table 1 gives a summary of the four major cell death mechanisms in NAFLD, highlighting their roles, triggers, key pathways, and potential therapeutic targets. Understanding these cell death mechanisms is crucial for developing targeted therapies. In the following sections, we will delve deeper into each of these cell death mechanisms, exploring their molecular pathways, roles in NAFLD progression, and the therapeutic strategies targeting these pathways.

Table 1: four types of cell death in the NAFLD progression

Type	Role in NAFLD	Triggers	Key Pathways	Therapies
Apoptosis	Early lipotoxic stage	FFAs, ER stress	Caspase-8/9, Bcl-2/Bax	Selenium, Vitamin D
Necroptosis	Early lipotoxic stage	TNF-α, RIPK3/MLKL	RIPK1-RIPK3-MLKL axis	GSK-872, Metformin
Pyroptosis	Amplification of inflammation and fibrosis	NLRP3 inflammasome	Caspase-1/GSDMD cleavage	MCC950, Baicalin
Ferroptosis	Oxidative stress and lipid peroxidation	Iron overload	GPX4 inactivation	DFO, Icariin

DOI: 10.54254/2753-8818/96/2025.22284

3.1. Apoptosis

Cell shrinking, chromosome condensation, and the development of apoptotic bodies are the features of apoptosis, a highly controlled type of programmed cell death. In NAFLD, hepatocyte apoptosis is a key driver of disease progression, particularly in the transition from steatosis to NASH. The intrinsic mechanism, which involves mitochondrial malfunction and cytochrome c release, or the extrinsic pathway, which is regulated by death receptors like Fas and TNF-α, can both cause apoptosis [11].

Studies have shown that FFAs and lipotoxicity induce hepatocyte apoptosis by activating caspase-8 and caspase-9, leading to the execution of apoptosis through caspase-3 [12]. Elevated levels of apoptotic markers, involving cleaved caspase-3 and TUNEL-positive cells, are observed in NAFLD patients, correlating with disease severity [13]. Therapeutic strategies targeting apoptosis include antioxidants like selenium and vitamin D, which reduce oxidative stress and inhibit apoptotic pathways. Additionally, compounds such as lentinan and γ-linolenic acid have been shown to modulate apoptosis by regulating the Bcl-2/Bax ratio and caspase activity [14].

3.2. Necroptosis

Necroptosis is a type of necrotic cell death regulated by the receptor-interacting kinases, which include RIPK1 and RIPK3, as well as the mixed lineage kinase domain-like protein (MLKL). Unlike apoptosis, necroptosis causes plasma membrane rupture and the release of damage-associated molecular patterns (DAMPs), exacerbating inflammation and fibrosis. In NAFLD, TNF-α signalling and lipotoxicity trigger necroptosis [15].

Elevated levels of RIPK3 and MLKL have been observed in NAFLD patients, particularly in those with advanced fibrosis [16]. Inhibition of necroptosis using RIPK1 inhibitors (e.g., necrostatin-1) or RIPK3 inhibitors (e.g., GSK-872) has been shown to reduce liver injury and inflammation in preclinical models [17]. These results point to necroptosis as a possible NAFLD treatment target.

3.3. Pyroptosis

The inflammasome activation gasdermin D (GSDMD) cleavage drive pyroptosis. Cell enlargement, membrane hole development, and the release of pro-inflammatory cytokines like IL-1β and IL-18 are its defining features. In NAFLD, pyroptosis is primarily mediated by the NLRP3 inflammasome, which is activated by lipotoxicity, mitochondrial dysfunction, and ER stress [18].

Hepatocyte pyroptosis and the production of inflammatory cytokines are the outcomes of caspase-1-dependent cleavage of GSDMD caused by activation of the NLRP3 inflammasome [19]. Hepatic fibrosis and inflammation are exacerbated by this process. NLRP3 inhibitors, for instance MCC950, and natural substances like antcin A, which prevent inflammasome activation and lessen pyroptosis, are examples of therapeutic approaches that target pyroptosis [20]. In NAFLD models, several methods have demonstrated potential in reducing liver fibrosis and damage.

3.4. Ferroptosis

Since Dixon's 2012 proposal, ferroptosis has been thoroughly investigated in a number of illnesses, including as malignancies, endocrine disorders, and neurodegenerative diseases [21]. Lipid peroxidation and glutathione peroxidase 4 (GPX4) inactivation is a hallmark of ferroptosis, a type of cell death that depends on iron. In NAFLD, ferroptosis is associated with iron overload and oxidative stress, which exacerbate hepatocyte damage and inflammation. Patients with NAFLD, especially those with NASH, have a greater amount of lipid peroxidation-related markers including malondialdehyde (MDA) [22].

DOI: 10.54254/2753-8818/96/2025.22284

Therapeutic approaches targeting ferroptosis include iron chelators (e.g., deferoxamine) and GPX4 activators (e.g., sodium selenite). These agents reduce lipid peroxidation and mitigate liver injury in preclinical models [23]. Additionally, natural compounds like icariin and puerarin have demonstrated anti-ferroptotic effects by activating the Nrf2 pathway and enhancing GPX4 expression [24]. According to these results, ferroptosis targeting may be an appropriate therapeutic approach for NAFLD.

4. Treatment

Current therapeutic strategies for NAFLD focus on addressing the underlying metabolic dysfunction, reducing inflammation, and preventing fibrosis. The cornerstone of NAFLD continues to be lifestyle policies, such as regular exercise and shedding pounds. Weight loss of 5-10% has been shown to reduce hepatic steatosis and improve liver histology [25].

Pharmacological therapies targeting metabolic pathways include insulin sensitizers such as pioglitazone and GLP-1 receptor agonists like liraglutide. Pioglitazone improves insulin sensitivity and reduces hepatic steatosis, while liraglutide promotes weight loss and has anti-inflammatory effects [26]. Antioxidant vitamin E has been demonstrated to enhance liver histology and lessen oxidative stress in non-diabetic NASH patients [27].

Emerging therapies targeting cell death pathways hold promise for NAFLD treatment. For example, caspase inhibitors like emricasan have been proven in preclinical experiments to decrease liver fibrosis and hepatocyte apoptosis [28]. Similarly, necroptosis inhibitors such as necrostatin-1 and GSK-872 have demonstrated efficacy in reducing liver injury and inflammation [29]. NLRP3 inflammasome inhibitors, including MCC950 and antcin A, have shown potential in alleviating pyroptosis and liver fibrosis [30].

Ferroptosis inhibitors, such as iron chelators and GPX4 activators, are also being explored for their therapeutic potential. Deferoxamine and sodium selenite have been shown to reduce lipid peroxidation and liver injury in NAFLD models [31]. Natural compounds like icariin and puerarin, which activate the Nrf2 pathway, have demonstrated anti-ferroptotic effects and improved liver function [32].

5. Conclusion

Non-alcoholic fatty liver disease (NAFLD) progression arises from interconnected metabolic dysfunction, oxidative stress, and inflammatory processes, with cell death pathways critically shaping disease severity. Apoptosis, characterized by regulated cellular self-destruction, acts as a pivotal indicator of disease advancement, particularly during the transition from simple steatosis to inflammatory NASH. Inflammatory cell death pathways like necroptosis and pyroptosis amplify liver damage by releasing damage-associated molecular patterns (DAMPs) and inflammatory cytokines, perpetuating tissue injury. Ferroptosis, marked by iron-dependent lipid peroxidation, has recently been recognized as a significant contributor to NAFLD pathogenesis through oxidative membrane damage. These interconnected cell death mechanisms collectively drive hepatocyte injury, inflammatory responses, and fibrotic remodeling in the liver.

Contemporary therapeutic approaches for NAFLD prioritize lifestyle adjustments and pharmacotherapy to ameliorate metabolic imbalances and attenuate inflammatory cascades. However, Novel therapeutic strategies directed at modulating cell death pathways—including caspase suppression, necroptosis blockade, and ferroptosis inhibition—exhibit potential to enhance clinical efficacy in NAFLD management. For example, caspase inhibitors like emricasan have shown efficacy in reducing hepatocyte apoptosis and liver fibrosis, while necroptosis inhibitors such as necrostatin-1 and GSK-872 have demonstrated potential in mitigating liver injury and inflammation.

DOI: 10.54254/2753-8818/96/2025.22284

NLRP3 inflammasome inhibitors, including MCC950 and antcin A, have shown promise in alleviating pyroptosis and liver fibrosis. Ferroptosis inhibitors, such as iron chelators and GPX4 activators, are also being explored for their therapeutic potential.

Despite substantial advances in understanding the processes of cell death in NAFLD, various hurdles still exist. The interactions of various cell death pathways and their functions in fibrosis regression are not fully understood. To boost therapeutic efficacy, multi-targeted medicines that address numerous pathways at the same time are also required. Future studies ought to concentrate on understanding the molecular processes that underpin these pathways and identifying novel treatment targets. The incorporation of traditional medicine and natural substances into NAFLD treatment regimens also has significant promise.

In conclusion, intervening in cell death signalling pathways offers a viable avenue for developing targeted NAFLD therapies. By addressing the underlying mechanisms of hepatocyte injury and inflammation, these therapies have the potential to halt disease progression and improve patient outcomes. Ongoing research and clinical trials are essential to translate these findings into effective treatments for NAFLD.

References

[1] *Luedde T., Kaplowitz N., Schwabe R.F. (2014) Cell Death and Cell Death Responses in Liver Disease: Mechanisms and Clinical Relevance. Gastroenterology, 147: 765-783.e4.*

[2] *Younossi Z.M., et al. (2016) Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology, 64(1): 73-84.*

[3] *Friedman S.L., et al. (2018) Mechanisms of NAFLD development and therapeutic strategies. Nature Medicine, 24(7): 908-922.*

[4] *Loomba R., et al. (2021) Mechanisms and disease consequences of nonalcoholic fatty liver disease. Cell, 184(10): 2537-2564.*

[5] *Dixon S.J., et al. (2012) Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell, 149(5): 1060-1072.*

[6] *Xu H., et al. (2024) Targeting cell death in NAFLD: mechanisms and targeted therapies. Cell Death Discovery, 10(1): 399.*

[7] *Powell E.E., et al. (2021) Non-alcoholic fatty liver disease. The Lancet, 397(10290): 2212-2224.*

[8] *Buzzetti E., et al. (2016) The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism, 65(8): 1038-1048.*

[9] *Tilg H., et al. (2010) Evolution of inflammation in nonalcoholic fatty liver disease: The multiple parallel hits hypothesis. Hepatology, 52(5): 1836-1846.*

[10] *Leung C., et al. (2016) The role of the gut microbiota in NAFLD. Nature Reviews Gastroenterology & Hepatology, 13(7): 412-425.*

[11] *Feldstein A.E., et al. (2003) Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. Gastroenterology, 125(2): 437-443.*

[12] *Malhi H., et al. (2006) Free fatty acids induce JNK-dependent hepatocyte lipoapoptosis. Journal of Biological Chemistry, 281(17): 12093-12101.*

[13] *Afonso M.B., et al. (2019) Processes exacerbating apoptosis in non-alcoholic steatohepatitis. Clinical Science, 133(20): 2245-2264.*

[14] *Du T., et al. (2022) Lentinan protects against nonalcoholic fatty liver disease by reducing oxidative stress and apoptosis via the PPARα pathway. Metabolites, 12(1): 55.*

[15] *Gautheron J., et al. (2015) Necroptosis in nonalcoholic steatohepatitis. Cellular and Molecular Gastroenterology and Hepatology, 1(3): 264-265.*

[16] *Afonso M.B., et al. (2015) Necroptosis is a key pathogenic event in human and experimental murine models of non-alcoholic steatohepatitis. Clinical Science, 129(8): 721-739.*

[17] *Majdi A., et al. (2020) Inhibition of receptor-interacting protein kinase 1 improves experimental non-alcoholic fatty liver disease. Journal of Hepatology, 72(4): 627-635.*

[18] *Wree A., et al. (2014) NLRP3 inflammasome activation results in hepatocyte pyroptosis, liver inflammation, and fibrosis in mice. Hepatology, 59(3): 898-910.*

[19] *Shi, J. et al. (2015) Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature, 526(7575): 660-665.*

DOI: 10.54254/2753-8818/96/2025.22284

[20] Ruan, S. et al. (2021) Antcin A alleviates pyroptosis and inflammatory response in Kupffer cells of non-alcoholic fatty liver disease by targeting NLRP3. International Immunopharmacology, 100: 108126.
[21] Yu, Q., Song, L. (2024) Unveiling the role of ferroptosis in the progression from NAFLD to NASH: recent advances in mechanistic understanding. Frontiers in Endocrinology, 15.
[22] Qi, J. et al. (2020) Ferroptosis affects the progression of nonalcoholic steatohepatitis via the modulation of lipid peroxidation–mediated cell death in mice. American Journal of Pathology, 190(1): 68-81.
[23] Yang, W.S. et al. (2014) Regulation of ferroptotic cancer cell death by GPX4. Cell, 156(1-2): 317-331.
[24] Yang, Y. et al. (2020) Study on the attenuated effect of Ginkgolide B on ferroptosis in high fat diet induced nonalcoholic fatty liver disease. Toxicology, 445: 13559.
[25] Vilar-Gomez, E. et al. (2015) Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. Gastroenterology, 149(2): 367-378.
[26] Armstrong, M.J. et al. (2016) Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. The Lancet, 387(10019): 679-690.
[27] Sanyal, A.J. et al. (2010) Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. New England Journal of Medicine, 362(18): 1675-1685.
[28] Barreyro, F.J. et al. (2014) The pan-caspase inhibitor emricasan (IDN-6556) decreases liver injury and fibrosis in a murine model of non-alcoholic steatohepatitis. Liver International, 35(3): S3-S6.
[29] Roychowdhury, S. et al. (2016) Receptor interacting protein 3 protects mice from high-fat diet–induced liver injury. Hepatology, 64(5): 1518-1533.
[30] Mridha, A.R. et al. (2017) NLRP3 inflammasome blockade reduces liver inflammation and fibrosis in experimental NASH in mice. Journal of Hepatology, 66(5): 1037-1046.
[31] Tsurusaki, S. et al. (2019) Hepatic ferroptosis plays an important role as the trigger for initiating inflammation in nonalcoholic steatohepatitis. Cell Death & Disease, 10(6): 449.
[32] Choi, J. et al. (2023) Icariin supplementation suppresses the markers of ferroptosis and attenuates the progression of nonalcoholic steatohepatitis in mice fed a methionine choline-deficient diet. International Journal of Molecular Sciences, 24(12): 12510.