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**Necroptosis contributes to non-alcoholic fatty liver disease pathoetiology with promising diagnostic and therapeutic functions**

Sun HJ *et al*. Necroptosis in NAFLD

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**Abstract**

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent type of chronic liver disease. However, the disease is underappreciated as a remarkable chronic disorder as there are rare managing strategies. Several studies have focused on determining NAFLD-caused hepatocyte death to elucidate the disease pathoetiology and suggest functional therapeutic and diagnostic options. Pyroptosis, ferroptosis, and necroptosis are the main subtypes of non-apoptotic regulated cell deaths (RCDs), each of which represents particular characteristics. Considering the complexity of the findings, the present study aimed to review these types of RCDs and their contribution to NAFLD progression, and subsequently discuss in detail the role of necroptosis in the pathoetiology, diagnosis, and treatment of the disease. The study revealed that necroptosis is involved in the occurrence of NAFLD and its progression towards steatohepatitis and cancer, hence it has potential in diagnostic and therapeutic approaches. Nevertheless, further studies are necessary.

**Key Words:** Nonalcoholic fatty liver disease; Apoptosis; Necroptosis; Cell death; Diagnosis; Treatment

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**Core Tip:** Hepatocyte death has been hypothesized as a major contributor to nonalcoholic fatty liver disease (NAFLD) progression, however, the role of regulated cell death (RCD) programs in NAFLD pathophysiology and their potential as diagnostic/therapeutic strategies has not been comprehensively discussed. The present study reviewed the participation of pyroptosis, ferroptosis, and necroptosis in the establishment of NAFLD and its progression toward steatohepatitis and cancer and discussed the potential RCDs in the diagnosis/treatment of the disease. Particularly, the present findings revealed that necroptosis significantly contributes to NAFLD occurrence and progress that may represent promising functions as diagnostic/therapeutic tools.

**INTRODUCTION**

Nonalcoholic fatty liver disease (NAFLD) is described as the most prevalent type of chronic liver disease worldwide[1]. NAFLD is considered a growing cause of end-stage hepatic disorders throughout the world and emerged as a pathoetiology of hepatocellular carcinoma (HCC) even when the underlying cirrhosis is absent[2,3]. In fact, the excessive accumulation of lipids in the hepatocytes (in the form of triglycerides, > 5% fat content in the liver; referred to as steatosis) of people consuming alcohol at low-risk amounts is the main characteristic of patients with NAFLD[4]. Clinically, the condition may be restricted to excessive liver fat, known as NAFL, or progress to necroinflammation and fibrosis, called non-alcoholic steatohepatitis (NASH), to NASH-cirrhosis, and eventually to HCC[5,6]. In Western countries, it is estimated that one-third of the general population is affected by NAFLD which is associated with excess body weight and diabetes mellitus. Moreover, the disease is highly prevalent in the Middle East and the rate of incidence is growing in the Asian subcontinent and the Far East nations[7-9]. Altogether, NAFLD has become the most common chronic liver disorder with a worldwide prevalence of around 25% of the adult population that is recognized to be closely and bidirectionally related to components of metabolic syndrome[9,10].

The most important challenge is the identification of people with NAFLD who are at the highest risk of developing liver-related complications. The burden of end-stage liver disease is estimated to increase two to three times globally by 2030[11]. Although NALFD is clinically accepted as the most rapidly increasing cause of liver-related mortality emerged as a significant cause of end-stage liver disease, HCC, and liver transplantation with a substantial health economic burden, NAFLD is underappreciated as a remarkable chronic disorder and there is a few numbers of managing strategies or policies[12,13]. In addition, even though a variety of ongoing studies have assumed several genetic/metabolic aberrations as the causes of NAFLD pathogenesis, the underlying mechanism by which the disease occurs and progresses remains unclear, making early laboratory diagnosis and effective treatment challenging[14-17].

Generally, a variety of unrecoverable intra- or extra-cellular perturbations capable of disrupting cellular survival affect cells by the activation of one of many signaling cascades, causing the death of cells and tissue damage[18-20]. The gross diversity in cell death programs has made these processes fall into two major categories including accidental cell death, and regulated cell death (RCD). Accidental cell death is described as a passive process in which uninspected necrosis is the main type, while RCD is an active process that includes a number of subtypes[18-21]. Specific molecular mechanisms are considered the initiating and propagating agents of RCD modalities with remarkable interactivity. In addition, each type of RCD, discussed later, represents distinct molecular, biochemical, functional, and morphological properties with particular pathophysiological consequences. Two main categories of RCDs consist of apoptotic and non-apoptotic cell death programs, which include apoptosis in the first subtype and necroptosis, pyroptosis, autophagy, and ferroptosis in the second one[18-20,22]. Fortunately, a large number of studies have recently focused on determining the process of hepatocyte cell death related to NAFLD in order to elucidate the etiology of the disease and suggest effective therapeutic and diagnostic options[23]. Considering the breadth and complexity of the findings, the present study has aimed to first provide an overview of the types of RCDs and the contribution of each one in the disease progression briefly, and then discuss in detail the role of necroptosis, a novel form of RCD, in the pathoetiology and treatment of the disease.

***RCDs contribute to the progression of NAFLD***

RCD is considered intrinsically associated with inflammatory disorders of hepatic tissue and is documented to be pivotal in governing the clinical consequences of liver disorder[24,25]. A plethora of evidence has revealed different forms of RCD pathways with increasingly identified correlations with NAFLD[23]. Significantly, the novel described forms of RCDs may co-exist simultaneously in diseases, and a number of them portion overlapping molecular processes that may function as a backup dying approach to provide the survival of an organism when a cellular threshold induced by death is established[26-28]. In fact, hepatocellular death could be triggered by metabolic, viral, toxic, and/or autoimmune mediators accompanied by inflammation and compensatory proliferation which frequently represent a close association with the development of cirrhosis, fibrosis, and HCC[29].

Conventionally, apoptosis was described as a strictly controlled pathway, as opposed to the passive form of cell death known as necrosis which is an irregulated and accidental form of cellular death[30]. The external forces cause irreparably cell injury the passive form of cell death occurs which is characterized by oncosis, a rapid organelle, and cytoplasmic swelling[31]. Moreover, cell membrane permeabilization followed by leakage of damage-associated molecular patterns (DAMPs) occurs that initiates the immune response[32]. Recently, a plethora of research highlights a variety of forms of RCD modalities such as autophagy, ferroptosis, pyroptosis, and necroptosis that represent similar main morphological characteristics with necrosis[33,34], however, are regulated molecular pathways and have well-defined processes (Table 1).

Necroptosis represents several similar molecular components with apoptosis, particularly the extrinsic pathway, hence it may be the best-understood form of regulated necrosis. Also, necroptosis provides the progression of cellular death when apoptosis is pathologically inhibited, which in turn could be assumed as a disease state in the hepatic tissue[4]. Nonetheless, other mentioned forms of RCD probably be significant in the progression of NAFLD as a proper characterization of RCD in the disease can lead to promising diagnostic and therapeutic options. The novelty of the field has led to the rapid progress of research, and recent studies sought to describe the association between the non-apoptotic forms of RCD and the progression of NAFLD. Although the focus of the current study has been on elucidating the contribution of necroptosis in the pathogenesis of NAFLD, it is essential to first acquainted with other forms of non-apoptotic RCDs and briefly discuss the involvement of each one in the development of the disease.

***An overview of pyroptosis***

Zychlinsky *et al*[35] initially reported pyroptosis in the 1990s and described a lytic form of cell death in macrophages infected by *Shigella flexneri*. Nevertheless, the ‘pyroptosis’ term emerged in 2001 as scientists revealed that the death of macrophages induced by *Salmonella* was dependent on caspase (CASP)-1[36]. Subsequently, the number of pyroptosis-related CASPs that are opposed to apoptosis-related CASPs has significantly elevated and includes CASP-1, CASP-11, and the human orthologs CASP-4 and CASP-5[35,36]. Surprisingly the well-known apoptotic effector CASP-3 is considered a pyroptotic CASP under specific conditions[37,38]. Primarily, this type of RCD occurs after intracellular pathogens cause infection leading to the formation of cell membrane pores dependent on CASP activity, swelling, cell rupture, and release of pro-inflammatory interleukins[39] such as interleukin (IL)-1β and IL-18[40-42].

The pore-forming gasdermin D (GSDMD) was characterized in 2015 contributing as the executioner of pyroptosis[43,44]. 31-KDa N-terminal GSDMDNT fragment is produced when GSDMD is cleaved by the action of CASP-1 and CASP-11. GSDMDNT exhibits intrinsic pore-forming activity. Moreover, the cleavage of GSDMD produces a 22-KDa C-terminal GSDMDCT fragment. This fragment attaches to GSDMDNT in order to inhibit the protein[43,44]. The upregulation of GSDMDNT alone is followed by the induction of pyroptosis, however, GSDMDCT blocks GSDMDNT-induced pyroptosis[43,44]. Importantly, it is documented that GSDMD belongs to a larger family of proteins consisting of GSDMA to GSDME (also known as DFNA5), and DFNB59[45]. Recent investigations revealed that GSDME functions as another pyroptosis executioner, that is capable of switching CASP-3-mediated apoptosis to pyroptosis[46,47]. The majority of GSDMs have been linked to the incidence and development of a variety of diseases, however, the exact molecular and functional activation mechanisms remain mainly unknown[48].

Two signaling pathways including canonical and non-canonical signalings activate pyroptosis. These two signalings differ in the application of cytoplasmic multiprotein complexes known as inflammasomes[49,50]. Inflammasomes trigger the canonical pathway of pyroptosis. In this regard, inflammasomes can recognize different endogenous and exogenous danger signals such as pathogen-associated molecular patterns (PAMPs) and DAMPs. The canonical inflammasomes consist of a sensor protein that belongs to the nucleotide-binding domain (NBD), apoptosis-associated speck-like protein containing a CARD (ASC), AIM2-like receptor or NLR (leucine-rich-repeat-containing) or pyrin family, and pro-CASP-1 which is an inactive zymogen[51]. When the canonical inflammasome is formed, the activation of CASP-1 leads to the cleavage of pro-IL-1β and pro-IL-18 into their active forms. Next, these two ILs are released extracellularly subsequent to the action of GSDMDNT that causes spore formation in the cell membrane. Whereas, the non-canonical pathway is CASP-11-dependent without inflammasome priming cleaves GSDMD. In this type of pyroptosis, GSDMDNT signals back to canonical NLRP3 inflammasome leading to the activation of the CASP-1-dependent pathway[52].

***Pyroptosis may contribute to NAFLD progression and transition to NASH***

A variety of factors such as lipotoxicity, mitochondrial dysfunction, endoplasmic reticulum stress, hepatocyte death pathways, and innate immune response are able to initiate chronic inflammatory processes in the liver that may provide fuel for the transition from NAFL to NASH[53-55]. The infiltration of macrophages and activation of local Kupffer cells is considered a key characteristic of disease pathoetiology[56]. Tumor necrosis factor (TNF)-α is released by Kupffer cells[57] which feeds a vicious cycle of inflammatory responses and initiates fibrosis after activating apoptosis. Nonetheless, it has recently appeared that inflammatory CASPs such as human CASP-4/5, CASP-1, and murine CASP-11 contribute pivotally as inflammation mediators[44]. Thereby, it is assumed that pyroptosis is crucially involved in NAFL development and progression to NASH.

It is documented that the activation of inflammasome by typical factors such as uric acid, DAMPs, and fatty acids, which increase the expression of NLRP3 components, could trigger the activity of CASPs, promoting inflammation, and causing liver fibrosis[58]. The excessive function of inflammation-related CASPs is implicated directly in the NAFLD pathoetiology, where key effector molecules are considered to be pro-inflammatory cytokines released meanwhile[59,60]. Moreover, the generic substrate for inflammatory CASPs, GSDMD, functions as a downstream mediator of the activation of non-canonical inflammasome by contributing to inflammatory CASP-mediated pyroptosis[61,62]. Importantly, the GSDMDNT domain representing intrinsic pyroptosis-inducing activity in patients with NASH was positively associated with the NAFLD activity score and fibrosis[63]. In fact, the lipogenic gene *Srebp1c* downregulation and upregulation of *Pparα*, a lipolytic gene, and its downstream targets, induced the protection of *Gsdmd*-/- animals from steatosis[63].

As mentioned earlier, DAMPs and PAMPs can cause pyroptosis-related hepatocyte death directly or indirectly causing hepatic damage. It is reported that animals with mutations in myeloid-specific *Nlrp3* do not reveal detectable pyroptotic-mediated hepatocyte death and represent less severe hematopoietic stem cell activation[64]. Hence, one can conclude that in addition to immune cells, pyroptosis in hepatocytes caused by the activation of intrinsic inflammasome can exacerbate inflammation and fibrosis in the liver, determining that both immune cell- and liver-specific NLRP3 inflammasome activation as essential contributors to liver injury[64,65]. However, it is required to investigate hepatocyte-specific *NLRP3* mutant animals to provide convincing evidence of the correlation between hepatocytes and pyroptosis in the onset and progression of liver injury in NAFLD.

***An overview of ferroptosis***

Ferroptosis was initially reported early 20th century by a cell-permeable compound called erastin, a compound which was lethal to cancer cells of humans with an oncogenic *RAS* mutation[66]. A decade later the term ferroptosis was established to describe an erastin-caused RCD mediated by the accumulation of lipid peroxides dependent on iron[67]. Ferroptosis exerts tumor-suppressor activities, increased mitochondrial membrane density, and cell shrinkage without any typical necrotic or apoptotic manifestations[67]. Similar to pyroptosis, two different signalings including canonical and noncanonical pathways are described as ferroptosis inducers. In the canonical pathway, the glutathione (GSH) peroxidase 4 (GPX4) enzyme is inactivated eighter directly or indirectly which induces ferroptosis, whereas, in a noncanonical manner, the labile iron pool is increased[67].

The direct interaction of erastin with the transporter solute carrier family 7 member 5 (SLC7A5) and subsequent disruption of amino acids transport into the cell by the Xc- system occurs in the canonical pathway of ferroptosis[67]. The regulatory SLC3A2 and a catalytic subunit SLC7A11 are components of the Xc- system that are responsible for the exchange of cystine with glutamate by elevated promotion of cystine cellular uptake[68]. Cystine is the plasma precursor of cysteine that is essential for the synthesis of GSH, a major redox regulatory system[69]. Therefore, the blockage of cystine by inhibitors (*e.g.,* erastin, L-glutamate, *etc.*) is followed by the inhibition of GSH synthesis, suppression of GPX4, and accumulation of phospholipid hydroperoxides (PL-OOH), considered the main mediator of lipoxygenases (LOXs) chain reactions[70].

In the state of intracellular free iron overload, it interacts with reactive oxygen species[71] which ultimately leads to the production of hydroxyl radical that is highly reactive to macromolecules such as polyunsaturated fatty acids (PUFAs)[72]. The oxidation of PUFAs *via* a pathway involving lysophosphatidylcholine acyltransferase 3, acyl-CoA synthetase long-chain family member 4, and LOXs is required for ferroptosis caused lipotoxicity[73-75]. GPX4 is considered the only member capable of reducing membrane phospholipid hydroperoxides determining its significant contribution to confronting permeabilization of plasma membrane, peroxidation of lipids, and ultimately release of DAMPs[76]. In the non-canonical pathway, oxidative damage and ferroptosis are promoted by elevated uptake of iron by transferrin receptor and reduced export of iron by ferroportin[77,78].

***Scarce information on the ferroptosis involvement in NAFLD progression***

Unfortunately, there is scarce evidence demonstrating the contribution of ferroptosis in NAFLD pathogenesis. However, malondialdehyde and 4-hydroxynonenal[71], as secondary lipid peroxidation products, are suggested as stress markers in patients with NASH[79]. In this regard, well-known antioxidants capable of suppressing lipid peroxidation such as vitamin E and quercetin[80,81] potentially could reduce the levels of alanine transferase in patients with NASH[82,83]. In addition, the accumulation of iron due to metabolic dysfunction is followed by the aggravation of NASH as liver cirrhosis was reported[39]. Similarly, the exacerbation of primary hemochromatosis is observed in patients with NASH and iron overload[84], while the removal of iron was accompanied by the amelioration of hepatic damage and alanine transferase levels[85]. Furthermore, evidence suggesting the role of ferroptosis in liver steatosis has been discussed[86,87]. Nevertheless, documented information regarding the role of ferroptosis in NAFLD deserves further investigation in appropriate patient models with the disorder, particularly since currently no exact therapeutic strategies are available.

The modification of ferroptosis is considered a novel therapeutic option to confront malignancies[88]. Tyrosine kinase inhibitors (TKIs) are described as the first approved systemic treatments for advanced HCC, however, the systemic treatment of HCC has been further developed with the immune checkpoint inhibitor[89]. Recent evidence suggests the treatment with atezolizumab plus bevacizumab over sorafenib. Fortunately, the latest findings have suggested novel therapeutic strategies based on RCD modifications to confront HCC[89,90]. Lenvatinib, a well-known TKI, could suppress HCC progression *via* the induction of ferroptosis through the inhibition of fibroblast growth factor receptor-4[91]. Metronomic capecitabine, as another example, has been suggested as a second-line therapy in HCC patients after sorafenib failure[92] or discontinuation[93]. Similarly, the study conducted by Wang *et al*[94] indicated the ability of metronomic capecitabine to induce ferroptosis in CD4+ T cells, which is probably attributed to autophagy-related GPX4 degradation in these immune cells, caused the amelioration of liver transplantation rejection[94]. Concordantly, artesunate is considered a well-tolerated and appropriate combination therapy that synergizes with sorafenib to promote ferroptosis in HCC cells[95]. Moreover, GSH S-transferase zeta 1, an enzyme involved in the catabolism of phenylalanine, can inhibit the NRF2/GPX4 axis leading to sensitizes HCC cells to sorafenib-induced ferroptosis[96]. Similarly, tiliroside induces ferroptosis *via* targeting TANK-binding kinase 1 leading to the death of sorafenib-resistant HCC cells[97]. Therefore, it appears necessary for further studies to address the effects of ferroptosis modulators on the death of HCC cells treated with chemotherapeutics.

***An overview of necroptosis***

Ray and Pickup[98] provided the first evidence of necroptosis in 1996 when they observed a lytic mode of pig kidney cell death infected with the cowpox virus governed by the expression of a CASP inhibitor known as the viral cytokine response modifier A. Four years later, Holler *et al*[71] revealed that the classical death receptors including FAS, TRAIL, and TNF receptors triggered cell death by two alternative pathways. One of these pathways relied on CASP-8, the classical extrinsic pathway of apoptosis, while the one that was dependent on the receptor-interacting protein kinase 1 (RIPK1), the necroptosis. Nevertheless, it was in 2005 when this mode of cell death was named as Degterev *et al*[99] demonstrated that a compound that inhibits the kinase activity of RIPK1, known as necrostatin-1, could inhibit the death of TNF-treated cell lines. Subsequently, the two downstream core components of the necroptotic machinery have been identified that are RIPK3 and mixed lineage kinase domain-like pseudokinase (MLKL)[100-102]. Necroptosis is primarily initiated after infections and stressors such as chemotherapy or radiation and morphologically exhibits the characteristics of necrosis, for example in response to extreme external factors with loss of membrane integrity, elevated cell volume, swelling of organelles, and cellular collapse. In addition, necroptosis is followed by the release of DAMPs such as IL-1α, high-mobility group box 1, and IL-33[103,104]. Specific DAMPs related to necroptosis have not been documented so far, however, the release of DAMPs during necroptosis provokes a severe inflammatory response associated with the development of several diseases[103,104].

The activation of death receptors (DRs) (*e.g.*, TRAIL-R, CD95, TNFR1) and the inactivation, inhibition, or absence of apoptosis signaling components are the two main preconditions for the initiation of necroptosis. In fact, the formation of the RIPK1/RIPK3 platform known as necrosome occurs upon the activation of DRs[20]. In the necrosome, RIPK1 and RIPK3 interact with critical RIP homotypic interaction motifs to adopt a hetero-amyloid structure[105]. Next, RIPK3 phosphorylates MLKL leading to the oligomerization and finally translocation of MLKL to the cell membrane[20]. Along with RIPK1, it is suggested that family proteins of casein kinase 1 as necrosome ingredients directly phosphorylate human RIPK3 to induce necroptosis[106]. Ultimately, pore formation in the cell membrane occurs upon MLKL translocation accompanied by the increment of permeability through activation of Ca2+ influx, metalloproteinase and A disintegrin, and phosphatidylserine externalization[107-109]. It is widely accepted that remarkable crosstalk regulations exist between necroptosis and apoptosis in DR-dependent cell death pathways[110], hence one cannot be activated without inhibiting the other. Concordantly, CASP-8 cleaves and inactivates RIPK3 and RIPK1 revealing that apoptosis initiation suppresses necroptosis[111], whereas the activity of RIPK3 determines whether cells die by necroptosis or apoptosis[111,112].

In addition to DRs, a variety of pathways such as nucleic acid sensors (*e.g.,* Z-DNA-binding protein 1, also known as DAI)[113], toll-like receptors (TLRs) such as TLR4 and TLR3[114], retinoic acid-inducible gene 1 protein[115], TNF[116], and adhesion receptors[117] could initiate necroptosis. However, these signaling pathways are frequently RIPK1-independent, although phosphorylation and activation of RIPK3 and MLKL are required[20]. It should be noted that a membrane remodeling and scission machinery known as the endosomal sorting complexes required for transport-III complexes can promote membrane repair and thereby limit MLKL-mediated necroptosis[118]. The contribution of MLKL to the regulation of endosomal trafficking and extracellular vesicle generation reveals a delicate balance between membrane injury and repair determining the ultimate cell fate in necroptosis[119].

***Necroptosis exerts a pivotal role in NAFLD***

A plethora of evidence has considered an increased level of necroptosis in human cells as one of the main events in the progression of different pathological states including NAFLD, which is generally accompanied by an increase in the infiltration of immune cells and the induction of inflammation[120-122]. Therefore, necroptosis can be assumed as a potential therapeutic target. In addition, the detection of patients with NAFLD and distinguishing it from pathological states with similarities in clinical manifestations and laboratory findings may be another merit of examining necroptosis in patients. In the following, the current study first discusses the participation of necroptosis in the pathoetiology of the disease and then critically reviews the possible application of necroptosis as a novel diagnostic and therapeutic strategy.

***Necroptosis contributes to the progression of the disease***

Shreds of evidence during the last two decades have suggested that necroptosis is involved in the occurrence of the disease and actively participates in the progression of NAFLD towards NASH and HCC. The occurrence of cell death in hepatocytes is assumed to be necessary for the occurrence and progression of NAFLD[123,124]. Accordingly, it has been documented that NAFLD coincides with the induction of inflammation, disruption of lipid homeostasis, and characteristics of metabolic syndrome[125], meanwhile, necroptosis is involved with inflammatory responses and intracellular bioenergetic regulation[126,127]. In this regard, the involvement of the RIPK3 in the mitochondrial bioenergetics of hepatocytes[128], the necroptotic death of white adipocytes in NAFLD patients[129], and the induction of necroptosis caused by oleic/palmitic acid imbalance in hepatocytes isolated from patients[130] with NAFLD could be assumed valid markers of the contribution of necroptosis to the lipid metabolism-dependent occurrence of NAFLD. In addition, the induction of necroptosis caused by inflammatory mediators such as TNF[131,132], TLR4[133], and IL-6[134] is considered another event involved in the occurrence of NAFLD.

In addition to inflammatory and metabolic mediators, other signaling pathways may initiate necroptosis and cause NAFLD occurrence. For instance, it has been demonstrated that polarity protein AF6 can directly interact with the intermediate domain of RIPK1 and regulate its ubiquitination mediated by the deubiquitylase enzyme USP21 leading to the promotion of necroptosis in hepatocytes[135]. In this regard, the overexpression of AF6 results in the TNFα-induced necroptosis-mediated mortality of liver cells while hepatocyte-specific deletion of AF6 suppressed necroptosis and the subsequent inflammation in different non-alcoholic liver diseases[135]. The prevention of NAFLD *via* restriction of MLKL-dependent necroptosis by epigenetic silencing of RIPK3 reveals that the initiation of necroptotic-mediated liver cell death contributes to the NAFLD occurrence[136]. In addition to necroptosis-related death, MLKL signaling is involved in NAFLD pathogenesis by regulating other cell death programs such as autophagic flux[137]. Forkhead box protein O1 (FOXO1) is another effective factor in inducing necroptosis and NAFLD where two distinct studies have covered this issue with a different approach. The first study by Qian *et al*[133] showed that Serpina3c deficiency induced necroptosis and NAFLD by FOXO1 overexpression, while the other assumed that FOXO1 induces necroptosis and endoplasmic reticulum stress, and as a result, is involved in the pathogenesis of the disease[138]. Similarly, it has been documented that necroptosis induced by oxidative stress plays a key role in the pathogenesis of NAFLD and subsequent liver fibrosis[139,140].

Disease progression towards NASH and HCC is one of the undesired consequences of necroptosis induction in NAFLD patients. Several studies have assumed that cross-talk between RCDs is involved in the promotion of inflammation and the establishment of NASH following NAFLD[87,137,141,142]. It has been repeatedly shown that the induction of inflammation (for example, through TNF and TLR) and the resulting necroptosis actively cause NAFLD-to-NASH transition[143,144]. Importantly, necroptosis has been described as a pathological event in the liver that facilitates the appearance of steatohepatitis, as it has been reported that RIPK1 and RIPK3 cause hepatocyte death and exacerbation of NASH by inducing inflammation within macrophages and interacting with the JNK pathway[145-147]. In addition, diet is one of the factors that may contribute to NAFLD progression and steatosis by modulating RIPK3, inflammation, and necroptosis[148,149]. Although rare evidence of necroptosis involvement in the NAFLD-to-HCC transition has been reported, two recent studies have clarified that RIPK3 as a regulator of lipid metabolism participates in liver carcinogenesis, and RIPK3/MLKL absence reduces the risk of carcinogenesis[150,151].

The active involvement of necroptosis in the occurrence and progression of the disease continues with its effect in determining the severity of hepatic tissue injury. For example, the exacerbation of liver injury due to myeloid deficiency of CCN3 is mediated through the activation of necroptosis[152]. Also, the crosstalk between necroptosis and inflammatory mediators promoted both necroptosis and inflammation in liver fatty cells and as a result, aggravated liver damage in NAFLD models[134,153,154]. SPARC overexpression is another pathological event that leads to severe liver damage in patients with NAFLD by increasing the level of RIPK1/RIPK3 and promoting necroptosis[155]. Importantly, the involvement of necroptosis in the pathogenesis of the disease as well as the aggravation of pathological consequences can promise it as a potential biomarker for NAFLD early detection, grading, and prediction of progression. Moreover, targeting upstream effectors that promote necroptosis is an interesting novel strategy that may be effective in disease management. Therefore, in the next two sections, the findings related to the diagnostic and therapeutic efficacy of necroptosis in patients with NAFLD have been reviewed (Table 2).

***The diagnostic value of necroptosis***

Although the involvement of necroptosis in the occurrence of NAFLD and its progression to NASH and HCC has been appropriately elucidated, rare reports of the diagnostic value of necroptosis in the early detection and grading of the disease have been presented. Considering the coincidence of necroptosis and inflammation and the strengthening effect they have on each other, the main markers presented have been related to inflammatory responses. For example, the evidence presented on the changed levels of TNF-α, IL-10, and IL-1α in MAFLD patients can be considered a potential diagnostic marker[134,156,157]. In addition, it has been demonstrated that the high level of TNF-α along with the low level of serum IL-10 can be an indicator of the severity of NAFLD in the morbidity of obese men[156], and these two markers have provided promising efficacy in the follow-up of patients with NAFLD[158]. Similarly, it has been suggested that polymorphisms in the gene encoding TNF-α may be a marker of NAFLD progression and risk of coronary artery disease[159]. Accordingly, a 4-year follow-up study has revealed that TNF-α can function as a predictor of NAFLD development[131]. Although TNF-α is one of the triggering factors of the necroptotic cell death pathway and its diagnostic ability can be assumed to be related to necroptosis, further studies to find innate markers of necroptosis appear necessary. Moreover, inflammatory markers are mainly expressed in a wide range of disorders, and the lack of reporting of the sensitivity and specificity of the proposed markers complicates the determination of their diagnostic value.

Pathological alterations in the expression of MLKL, RIPK1, and RIPK3 can be suggested as the most potential markers related to necroptosis in diagnosing NAFLD and predicting its progress[136,150,160,161]. A recent study has determined that metabolomic and lipidomic screening has identified the participation of RCDs, particularly necroptosis, in the progression of NAFLD toward cancer[162], which may be of possible clinical importance in the follow-up of patients and determining the risk of disease progression. Nevertheless, the available evidence is rare, therefore further studies on this content represent a crucial necessity.

***Necroptosis as a potential therapeutic target***

It was previously discussed that the induction of necroptosis in hepatocytes is related to the occurrence and progression of NAFLD, therefore this pathway of cell death displays the characteristics of a therapeutic target. Several studies have investigated the efficiency of such properties, which can be reviewed in two categories, one contains the direct inhibitors of the mediators of the necroptosis pathway, and the other includes the herbals/chemicals that have revealed therapeutic properties through modulation of necroptosis.

It has been demonstrated that a highly specific inhibitor of RIPK1 known as RIPA-56 was able to ameliorate an animal model of NAFLD *via* down-regulation of MLKL, reduction of hepatic damage, inflammation, fibrosis, characteristic of NASH, as well as of steatosis[163]. In addition, the inhibition of MLKL resulted in a reduction of fat *de novo* synthesis and chemokine ligand expression in patients with NAFLD[164]. Similarly, chemical compounds that inhibited necroptosis along with apoptosis in hepatocytes were able to alleviate NAFLD-related characteristics[165-168]. Due to the cross-talk between RCDs, it is clinically important to note that the process of necroptosis inhibition should not activate other cell death pathways, as the absence of RIPK3 increased inflammation and hepatocyte apoptosis as well as early fibrotic responses leading to exacerbation of the disease[169].

Interestingly, a variety of herbal compounds such as epigallocatechin gallate, pentoxifylline, kaempferol, quercetin, metformin, *etc.* demonstrated anti-necroptotic properties that benefited the alleviation of NAFLD[170-174]. Regulating lipid metabolism, suppressing destructive inflammatory responses, maintaining cellular homeostasis, and also the rarity of adverse effects are attractive properties that antioxidant compounds provide in the treatment of NAFLD, in addition to inhibiting necroptosis. However, no clinical trials have been registered on the clinicaltrials.gov website, which indicates insufficient current information to confirm treatment strategies based on inhibition of necroptosis. Therefore, the conduct of further studies on this content is pivotally encouraged.

**CONCLUSION**

The findings of the current review revealed that the induction of necroptosis along with inflammatory responses pivotally contributes to the occurrence of NAFLD. Moreover, the continuation of the necroptotic death of hepatocytes can cause the disease to progress to NASH and HCC. Nevertheless, the diagnostic value of necroptosis-based markers has been rarely evaluated and disease management strategies based on necroptosis necessarily require further investigations in this direction.

**REFERENCES**

1 **Younossi ZM**, Golabi P, Paik JM, Henry A, Van Dongen C, Henry L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology* 2023; **77**: 1335-1347 [PMID: 36626630 DOI: 10.1097/HEP.0000000000000004]

2 **Estes C**, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology* 2018; **67**: 123-133 [PMID: 28802062 DOI: 10.1002/hep.29466]

3 **Mosavat SH**. Efficacy of traditional Persian medicine-based diet on non-alcoholic fatty liver disease: a randomized, controlled, clinical trial. *Galen Med J* 2017; **6**: 208-216 [DOI: 10.22086/gmj.v6i3.813]

4 **Ludwig J**, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980; **55**: 434-438 [PMID: 7382552]

5 **Geh D**, Anstee QM, Reeves HL. NAFLD-Associated HCC: Progress and Opportunities. *J Hepatocell Carcinoma* 2021; **8**: 223-239 [PMID: 33854987 DOI: 10.2147/JHC.S272213]

6 **Michelotti GA**, Machado MV, Diehl AM. NAFLD, NASH and liver cancer. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 656-665 [PMID: 24080776 DOI: 10.1038/nrgastro.2013.183]

7 **Farrell GC**, Wong VW, Chitturi S. NAFLD in Asia--as common and important as in the West. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 307-318 [PMID: 23458891 DOI: 10.1038/nrgastro.2013.34]

8 **Younossi ZM**, Blissett D, Blissett R, Henry L, Stepanova M, Younossi Y, Racila A, Hunt S, Beckerman R. The economic and clinical burden of nonalcoholic fatty liver disease in the United States and Europe. *Hepatology* 2016; **64**: 1577-1586 [PMID: 27543837 DOI: 10.1002/hep.28785]

9 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]

10 **Younossi Z**, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 11-20 [PMID: 28930295 DOI: 10.1038/nrgastro.2017.109]

11 **Estes C**, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, Caballeria J, Colombo M, Craxi A, Crespo J, Day CP, Eguchi Y, Geier A, Kondili LA, Kroy DC, Lazarus JV, Loomba R, Manns MP, Marchesini G, Nakajima A, Negro F, Petta S, Ratziu V, Romero-Gomez M, Sanyal A, Schattenberg JM, Tacke F, Tanaka J, Trautwein C, Wei L, Zeuzem S, Razavi H. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. *J Hepatol* 2018; **69**: 896-904 [PMID: 29886156 DOI: 10.1016/j.jhep.2018.05.036]

12 **Alexander M**, Loomis AK, Fairburn-Beech J, van der Lei J, Duarte-Salles T, Prieto-Alhambra D, Ansell D, Pasqua A, Lapi F, Rijnbeek P, Mosseveld M, Avillach P, Egger P, Kendrick S, Waterworth DM, Sattar N, Alazawi W. Real-world data reveal a diagnostic gap in non-alcoholic fatty liver disease. *BMC Med* 2018; **16**: 130 [PMID: 30099968 DOI: 10.1186/s12916-018-1103-x]

13 **Wong RJ**, Cheung R, Ahmed A. Nonalcoholic steatohepatitis is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma in the U.S. *Hepatology* 2014; **59**: 2188-2195 [PMID: 24375711 DOI: 10.1002/hep.26986]

14 **Cleveland E**, Bandy A, VanWagner LB. Diagnostic challenges of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Clin Liver Dis (Hoboken)* 2018; **11**: 98-104 [PMID: 30147867 DOI: 10.1002/cld.716]

15 **Roeb E**. Diagnostic and Therapy of Nonalcoholic Fatty Liver Disease: A Narrative Review. *Visc Med* 2022; **38**: 126-132 [PMID: 35614896 DOI: 10.1159/000519611]

16 **Wong VWS**, Zelber-Sagi S, Cusi K, Carrieri P, Wright E, Crespo J, Lazarus JV. Management of NAFLD in primary care settings. *Liver Int* 2022; **42**: 2377-2389 [PMID: 35986897 DOI: 10.1111/liv.15404]

17 **Zou YG**, Wang H, Li WW, Dai DL. Challenges in pediatric inherited/metabolic liver disease: Focus on the disease spectrum, diagnosis and management of relatively common disorders. *World J Gastroenterol* 2023; **29**: 2114-2126 [PMID: 37122598 DOI: 10.3748/wjg.v29.i14.2114]

18 **Ashkenazi A**, Salvesen G. Regulated cell death: signaling and mechanisms. *Annu Rev Cell Dev Biol* 2014; **30**: 337-356 [PMID: 25150011 DOI: 10.1146/annurev-cellbio-100913-013226]

19 **Christgen S**, Tweedell RE, Kanneganti TD. Programming inflammatory cell death for therapy. *Pharmacol Ther* 2022; **232**: 108010 [PMID: 34619283 DOI: 10.1016/j.pharmthera.2021.108010]

20 **Tang D**, Kang R, Berghe TV, Vandenabeele P, Kroemer G. The molecular machinery of regulated cell death. *Cell Res* 2019; **29**: 347-364 [PMID: 30948788 DOI: 10.1038/s41422-019-0164-5]

21 **Jaeschke H**, Ramachandran A. Acetaminophen-induced apoptosis: Facts versus fiction. *J Clin Transl Res* 2020; **6**: 36-47 [PMID: 33426354]

22 **Samare-Najaf M**, Neisy A, Samareh A, Moghadam D, Jamali N, Zarei R, Zal F. The constructive and destructive impact of autophagy on both genders' reproducibility, a comprehensive review. *Autophagy* 2023; **19**: 3033-3061 [PMID: 37505071 DOI: 10.1080/15548627.2023.2238577]

23 **Xiao Z**, Liu M, Yang F, Liu G, Liu J, Zhao W, Ma S, Duan Z. Programmed cell death and lipid metabolism of macrophages in NAFLD. *Front Immunol* 2023; **14**: 1118449 [PMID: 36742318 DOI: 10.3389/fimmu.2023.1118449]

24 **Gautheron J**, Gores GJ, Rodrigues CMP. Lytic cell death in metabolic liver disease. *J Hepatol* 2020; **73**: 394-408 [PMID: 32298766 DOI: 10.1016/j.jhep.2020.04.001]

25 **Clarke JI**, Brillanf N, Antoine DJ. Novel circulating- and imaging-based biomarkers to enhance the mechanistic understanding of human drug-induced liver injury. *J Clin Transl Res* 2017; **3**: 199-211 [PMID: 30873474]

26 **Ajoolabady A**, Tang D, Kroemer G, Ren J. Ferroptosis in hepatocellular carcinoma: mechanisms and targeted therapy. *Br J Cancer* 2023; **128**: 190-205 [PMID: 36229582 DOI: 10.1038/s41416-022-01998-x]

27 **Gibellini L**, Moro L. Programmed Cell Death in Health and Disease. *Cells* 2021; **10** [PMID: 34359935 DOI: 10.3390/cells10071765]

28 **Schwabe RF**, Luedde T. Apoptosis and necroptosis in the liver: a matter of life and death. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 738-752 [PMID: 30250076 DOI: 10.1038/s41575-018-0065-y]

29 **Wang K**. Molecular mechanisms of hepatic apoptosis. *Cell Death Dis* 2014; **5**: e996 [PMID: 24434519 DOI: 10.1038/cddis.2013.499]

30 **Kim-Campbell N**, Gomez H, Bayir H. Cell death pathways: apoptosis and regulated necrosis. In: Critical care nephrology (Third Edition). Amsterdam: Elsevier, 2019: 113-121.e112

31 **Zhang Q**, Jia M, Wang Y, Wang Q, Wu J. Cell Death Mechanisms in Cerebral Ischemia-Reperfusion Injury. *Neurochem Res* 2022; **47**: 3525-3542 [PMID: 35976487 DOI: 10.1007/s11064-022-03697-8]

32 **Lukenaite B**, Griciune E, Leber B, Strupas K, Stiegler P, Schemmer P. Necroptosis in Solid Organ Transplantation: A Literature Overview. *Int J Mol Sci* 2022; **23** [PMID: 35409037 DOI: 10.3390/ijms23073677]

33 **Galluzzi L**, Myint M. Cell death and senescence. *J Transl Med* 2023; **21**: 425 [PMID: 37386590 DOI: 10.1186/s12967-023-04297-y]

34 **Shen J**, San W, Zheng Y, Zhang S, Cao D, Chen Y, Meng G. Different types of cell death in diabetic endothelial dysfunction. *Biomed Pharmacother* 2023; **168**: 115802 [PMID: 37918258 DOI: 10.1016/j.biopha.2023.115802]

35 **Zychlinsky A**, Prevost MC, Sansonetti PJ. Shigella flexneri induces apoptosis in infected macrophages. *Nature* 1992; **358**: 167-169 [PMID: 1614548 DOI: 10.1038/358167a0]

36 **Cookson BT**, Brennan MA. Pro-inflammatory programmed cell death. *Trends Microbiol* 2001; **9**: 113-114 [PMID: 11303500 DOI: 10.1016/s0966-842X(00)01936-3]

37 **Ma Y**, Zhao R, Guo H, Tong Q, Langdon WY, Liu W, Zhang J, Zhang J. Cytosolic LPS-induced caspase-11 oligomerization and activation is regulated by extended synaptotagmin 1. *Cell Rep* 2023; **42**: 112726 [PMID: 37393619 DOI: 10.1016/j.celrep.2023.112726]

38 **Song H**, Yang B, Li Y, Qian A, Kang Y, Shan X. Focus on the Mechanisms and Functions of Pyroptosis, Inflammasomes, and Inflammatory Caspases in Infectious Diseases. *Oxid Med Cell Longev* 2022; **2022**: 2501279 [PMID: 35132346 DOI: 10.1155/2022/2501279]

39 **Nelson JE**, Wilson L, Brunt EM, Yeh MM, Kleiner DE, Unalp-Arida A, Kowdley KV; Nonalcoholic Steatohepatitis Clinical Research Network. Relationship between the pattern of hepatic iron deposition and histological severity in nonalcoholic fatty liver disease. *Hepatology* 2011; **53**: 448-457 [PMID: 21274866 DOI: 10.1002/hep.24038]

40 **Hachim MY**, Khalil BA, Elemam NM, Maghazachi AA. Pyroptosis: The missing puzzle among innate and adaptive immunity crosstalk. *J Leukoc Biol* 2020; **108**: 323-338 [PMID: 32083338 DOI: 10.1002/JLB.3MIR0120-625R]

41 **Huang X**, Feng Y, Xiong G, Whyte S, Duan J, Yang Y, Wang K, Yang S, Geng Y, Ou Y, Chen D. Caspase-11, a specific sensor for intracellular lipopolysaccharide recognition, mediates the non-canonical inflammatory pathway of pyroptosis. *Cell Biosci* 2019; **9**: 31 [PMID: 30962873 DOI: 10.1186/s13578-019-0292-0]

42 **Shariati A**, Raberi VS, Masumi M, Tarbiat A, Rastgoo E, Faramarzzadeh R. The Regulation of Pyroptosis and Ferroptosis by MicroRNAs in Cardiovascular Diseases. *Galen Med J* 2023; **12**: e2933 [DOI: 10.31661/gmj.v12i.2933]

43 **Kayagaki N**, Stowe IB, Lee BL, O'Rourke K, Anderson K, Warming S, Cuellar T, Haley B, Roose-Girma M, Phung QT, Liu PS, Lill JR, Li H, Wu J, Kummerfeld S, Zhang J, Lee WP, Snipas SJ, Salvesen GS, Morris LX, Fitzgerald L, Zhang Y, Bertram EM, Goodnow CC, Dixit VM. Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature* 2015; **526**: 666-671 [PMID: 26375259 DOI: 10.1038/nature15541]

44 **Shi J**, Zhao Y, Wang K, Shi X, Wang Y, Huang H, Zhuang Y, Cai T, Wang F, Shao F. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* 2015; **526**: 660-665 [PMID: 26375003 DOI: 10.1038/nature15514]

45 **Orning P**, Lien E, Fitzgerald KA. Gasdermins and their role in immunity and inflammation. *J Exp Med* 2019; **216**: 2453-2465 [PMID: 31548300 DOI: 10.1084/jem.20190545]

46 **Jiang M**, Qi L, Li L, Li Y. The caspase-3/GSDME signal pathway as a switch between apoptosis and pyroptosis in cancer. *Cell Death Discov* 2020; **6**: 112 [PMID: 33133646 DOI: 10.1038/s41420-020-00349-0]

47 **Shen X**, Wang H, Weng C, Jiang H, Chen J. Caspase 3/GSDME-dependent pyroptosis contributes to chemotherapy drug-induced nephrotoxicity. *Cell Death Dis* 2021; **12**: 186 [PMID: 33589596 DOI: 10.1038/s41419-021-03458-5]

48 **Broz P**, Pelegrín P, Shao F. The gasdermins, a protein family executing cell death and inflammation. *Nat Rev Immunol* 2020; **20**: 143-157 [PMID: 31690840 DOI: 10.1038/s41577-019-0228-2]

49 **Gan C**, Cai Q, Tang C, Gao J. Inflammasomes and Pyroptosis of Liver Cells in Liver Fibrosis. *Front Immunol* 2022; **13**: 896473 [PMID: 35707547 DOI: 10.3389/fimmu.2022.896473]

50 **Sun P**, Zhong J, Liao H, Loughran P, Mulla J, Fu G, Tang D, Fan J, Billiar TR, Gao W, Scott MJ. Hepatocytes Are Resistant to Cell Death From Canonical and Non-Canonical Inflammasome-Activated Pyroptosis. *Cell Mol Gastroenterol Hepatol* 2022; **13**: 739-757 [PMID: 34890842 DOI: 10.1016/j.jcmgh.2021.11.009]

51 **Vanaja SK**, Rathinam VA, Fitzgerald KA. Mechanisms of inflammasome activation: recent advances and novel insights. *Trends Cell Biol* 2015; **25**: 308-315 [PMID: 25639489 DOI: 10.1016/j.tcb.2014.12.009]

52 **Wright SS**, Vasudevan SO, Rathinam VA. Mechanisms and Consequences of Noncanonical Inflammasome-Mediated Pyroptosis. *J Mol Biol* 2022; **434**: 167245 [PMID: 34537239 DOI: 10.1016/j.jmb.2021.167245]

53 **Armandi A**, Bugianesi E. Natural history of NASH. *Liver Int* 2021; **41** Suppl 1: 78-82 [PMID: 34155792 DOI: 10.1111/liv.14910]

54 **Dong J**, Viswanathan S, Adami E, Singh BK, Chothani SP, Ng B, Lim WW, Zhou J, Tripathi M, Ko NSJ, Shekeran SG, Tan J, Lim SY, Wang M, Lio PM, Yen PM, Schafer S, Cook SA, Widjaja AA. Hepatocyte-specific IL11 cis-signaling drives lipotoxicity and underlies the transition from NAFLD to NASH. *Nat Commun* 2021; **12**: 66 [PMID: 33397952 DOI: 10.1038/s41467-020-20303-z]

55 **Velliou RI**, Legaki AI, Nikolakopoulou P, Vlachogiannis NI, Chatzigeorgiou A. Liver endothelial cells in NAFLD and transition to NASH and HCC. *Cell Mol Life Sci* 2023; **80**: 314 [PMID: 37798474 DOI: 10.1007/s00018-023-04966-7]

56 **Baffy G**. Kupffer cells in non-alcoholic fatty liver disease: the emerging view. *J Hepatol* 2009; **51**: 212-223 [PMID: 19447517 DOI: 10.1016/j.jhep.2009.03.008]

57 **Tosello-Trampont AC**, Landes SG, Nguyen V, Novobrantseva TI, Hahn YS. Kuppfer cells trigger nonalcoholic steatohepatitis development in diet-induced mouse model through tumor necrosis factor-α production. *J Biol Chem* 2012; **287**: 40161-40172 [PMID: 23066023 DOI: 10.1074/jbc.M112.417014]

58 **Szabo G**, Petrasek J. Inflammasome activation and function in liver disease. *Nat Rev Gastroenterol Hepatol* 2015; **12**: 387-400 [PMID: 26055245 DOI: 10.1038/nrgastro.2015.94]

59 **Beier JI**, Banales JM. Pyroptosis: An inflammatory link between NAFLD and NASH with potential therapeutic implications. *J Hepatol* 2018; **68**: 643-645 [PMID: 29408544 DOI: 10.1016/j.jhep.2018.01.017]

60 **Bessone F**, Razori MV, Roma MG. Molecular pathways of nonalcoholic fatty liver disease development and progression. *Cell Mol Life Sci* 2019; **76**: 99-128 [PMID: 30343320 DOI: 10.1007/s00018-018-2947-0]

61 **Kamajaya LJ**, Boucher D. Gasdermin D Cleavage Assay Following Inflammasome Activation. *Methods Mol Biol* 2022; **2459**: 39-49 [PMID: 35212952 DOI: 10.1007/978-1-0716-2144-8\_4]

62 **Yang R**. Interaction between caspases and their substrates in the inflammasome signaling pathway. [cited 15 November 2023]. Available from: https://etd.ohiolink.edu/acprod/odb\_etd/etd/r/1501/10?clear=10&p10\_accession\_num=case1559917811566556

63 **Xu B**, Jiang M, Chu Y, Wang W, Chen D, Li X, Zhang Z, Zhang D, Fan D, Nie Y, Shao F, Wu K, Liang J. Gasdermin D plays a key role as a pyroptosis executor of non-alcoholic steatohepatitis in humans and mice. *J Hepatol* 2018; **68**: 773-782 [PMID: 29273476 DOI: 10.1016/j.jhep.2017.11.040]

64 **Wree A**, Eguchi A, McGeough MD, Pena CA, Johnson CD, Canbay A, Hoffman HM, Feldstein AE. NLRP3 inflammasome activation results in hepatocyte pyroptosis, liver inflammation, and fibrosis in mice. *Hepatology* 2014; **59**: 898-910 [PMID: 23813842 DOI: 10.1002/hep.26592]

65 **Wree A**, McGeough MD, Inzaugarat ME, Eguchi A, Schuster S, Johnson CD, Peña CA, Geisler LJ, Papouchado BG, Hoffman HM, Feldstein AE. NLRP3 inflammasome driven liver injury and fibrosis: Roles of IL-17 and TNF in mice. *Hepatology* 2018; **67**: 736-749 [PMID: 28902427 DOI: 10.1002/hep.29523]

66 **Dolma S**, Lessnick SL, Hahn WC, Stockwell BR. Identification of genotype-selective antitumor agents using synthetic lethal chemical screening in engineered human tumor cells. *Cancer Cell* 2003; **3**: 285-296 [PMID: 12676586 DOI: 10.1016/s1535-6108(03)00050-3]

67 **Dixon SJ**, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, Morrison B 3rd, Stockwell BR. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 2012; **149**: 1060-1072 [PMID: 22632970 DOI: 10.1016/j.cell.2012.03.042]

68 **Lo M**, Wang YZ, Gout PW. The x(c)- cystine/glutamate antiporter: a potential target for therapy of cancer and other diseases. *J Cell Physiol* 2008; **215**: 593-602 [PMID: 18181196 DOI: 10.1002/jcp.21366]

69 **Lu SC**. Glutathione synthesis. *Biochim Biophys Acta* 2013; **1830**: 3143-3153 [PMID: 22995213 DOI: 10.1016/j.bbagen.2012.09.008]

70 **Yang WS**, SriRamaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS, Cheah JH, Clemons PA, Shamji AF, Clish CB, Brown LM, Girotti AW, Cornish VW, Schreiber SL, Stockwell BR. Regulation of ferroptotic cancer cell death by GPX4. *Cell* 2014; **156**: 317-331 [PMID: 24439385 DOI: 10.1016/j.cell.2013.12.010]

71 **Holler N**, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S, Bodmer JL, Schneider P, Seed B, Tschopp J. Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. *Nat Immunol* 2000; **1**: 489-495 [PMID: 11101870 DOI: 10.1038/82732]

72 **Feng H**, Stockwell BR. Unsolved mysteries: How does lipid peroxidation cause ferroptosis? *PLoS Biol* 2018; **16**: e2006203 [PMID: 29795546 DOI: 10.1371/journal.pbio.2006203]

73 **Doll S**, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, Ingold I, Irmler M, Beckers J, Aichler M, Walch A, Prokisch H, Trümbach D, Mao G, Qu F, Bayir H, Füllekrug J, Scheel CH, Wurst W, Schick JA, Kagan VE, Angeli JP, Conrad M. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat Chem Biol* 2017; **13**: 91-98 [PMID: 27842070 DOI: 10.1038/nchembio.2239]

74 **Kagan VE**, Mao G, Qu F, Angeli JP, Doll S, Croix CS, Dar HH, Liu B, Tyurin VA, Ritov VB, Kapralov AA, Amoscato AA, Jiang J, Anthonymuthu T, Mohammadyani D, Yang Q, Proneth B, Klein-Seetharaman J, Watkins S, Bahar I, Greenberger J, Mallampalli RK, Stockwell BR, Tyurina YY, Conrad M, Bayır H. Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nat Chem Biol* 2017; **13**: 81-90 [PMID: 27842066 DOI: 10.1038/nchembio.2238]

75 **Yang WS**, Kim KJ, Gaschler MM, Patel M, Shchepinov MS, Stockwell BR. Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. *Proc Natl Acad Sci U S A* 2016; **113**: E4966-E4975 [PMID: 27506793 DOI: 10.1073/pnas.1603244113]

76 **Xie Y**, Kang R, Klionsky DJ, Tang D. GPX4 in cell death, autophagy, and disease. *Autophagy* 2023; **19**: 2621-2638 [PMID: 37272058 DOI: 10.1080/15548627.2023.2218764]

77 **Kang YP**, Mockabee-Macias A, Jiang C, Falzone A, Prieto-Farigua N, Stone E, Harris IS, DeNicola GM. Non-canonical Glutamate-Cysteine Ligase Activity Protects against Ferroptosis. *Cell Metab* 2021; **33**: 174-189.e7 [PMID: 33357455 DOI: 10.1016/j.cmet.2020.12.007]

78 **Wang X**, Wang Y, Li Z, Qin J, Wang P. Regulation of Ferroptosis Pathway by Ubiquitination. *Front Cell Dev Biol* 2021; **9**: 699304 [PMID: 34485285 DOI: 10.3389/fcell.2021.699304]

79 **Loguercio C**, De Girolamo V, de Sio I, Tuccillo C, Ascione A, Baldi F, Budillon G, Cimino L, Di Carlo A, Di Marino MP, Morisco F, Picciotto F, Terracciano L, Vecchione R, Verde V, Del Vecchio Blanco C. Non-alcoholic fatty liver disease in an area of southern Italy: main clinical, histological, and pathophysiological aspects. *J Hepatol* 2001; **35**: 568-574 [PMID: 11690701 DOI: 10.1016/S0168-8278(01)00192-1]

80 **Jafari Khorchani M**, Samare-Najaf M, Abbasi A, Vakili S, Zal F. Effects of quercetin, vitamin E, and estrogen on Metabolic-Related factors in uterus and serum of ovariectomized rat models. *Gynecol Endocrinol* 2021; **37**: 764-768 [PMID: 33525940 DOI: 10.1080/09513590.2021.1879784]

81 **Samare-Najaf M**, Zal F, Jamali N, Vakili S, Khodabandeh Z. Do Quercetin and Vitamin E Properties Preclude Doxorubicin-induced Stress and Inflammation in Reproductive Tissues? *Curr Cancer Ther Rev* 2022; **18** [DOI: 10.2174/1573394718666220726105843]

82 **Gnoni A**, Di Chiara Stanca B, Giannotti L, Gnoni GV, Siculella L, Damiano F. Quercetin Reduces Lipid Accumulation in a Cell Model of NAFLD by Inhibiting De Novo Fatty Acid Synthesis through the Acetyl-CoA Carboxylase 1/AMPK/PP2A Axis. *Int J Mol Sci* 2022; **23** [PMID: 35162967 DOI: 10.3390/ijms23031044]

83 **Sanyal AJ**, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR; NASH CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; **362**: 1675-1685 [PMID: 20427778 DOI: 10.1056/NEJMoa0907929]

84 **Bonkovsky HL**, Jawaid Q, Tortorelli K, LeClair P, Cobb J, Lambrecht RW, Banner BF. Non-alcoholic steatohepatitis and iron: increased prevalence of mutations of the HFE gene in non-alcoholic steatohepatitis. *J Hepatol* 1999; **31**: 421-429 [PMID: 10488699 DOI: 10.1016/s0168-8278(99)80032-4]

85 **Valenti L**, Moscatiello S, Vanni E, Fracanzani AL, Bugianesi E, Fargion S, Marchesini G. Venesection for non-alcoholic fatty liver disease unresponsive to lifestyle counselling--a propensity score-adjusted observational study. *QJM* 2011; **104**: 141-149 [PMID: 20851820 DOI: 10.1093/qjmed/hcq170]

86 **Hernández-Alvarez MI**, Sebastián D, Vives S, Ivanova S, Bartoccioni P, Kakimoto P, Plana N, Veiga SR, Hernández V, Vasconcelos N, Peddinti G, Adrover A, Jové M, Pamplona R, Gordaliza-Alaguero I, Calvo E, Cabré N, Castro R, Kuzmanic A, Boutant M, Sala D, Hyotylainen T, Orešič M, Fort J, Errasti-Murugarren E, Rodrígues CMP, Orozco M, Joven J, Cantó C, Palacin M, Fernández-Veledo S, Vendrell J, Zorzano A. Deficient Endoplasmic Reticulum-Mitochondrial Phosphatidylserine Transfer Causes Liver Disease. *Cell* 2019; **177**: 881-895.e17 [PMID: 31051106 DOI: 10.1016/j.cell.2019.04.010]

87 **Tsurusaki S**, Tsuchiya Y, Koumura T, Nakasone M, Sakamoto T, Matsuoka M, Imai H, Yuet-Yin Kok C, Okochi H, Nakano H, Miyajima A, Tanaka M. Hepatic ferroptosis plays an important role as the trigger for initiating inflammation in nonalcoholic steatohepatitis. *Cell Death Dis* 2019; **10**: 449 [PMID: 31209199 DOI: 10.1038/s41419-019-1678-y]

88 **Samare-Najaf M**, Samareh A, Savardashtaki A, Khajehyar N, Tajbakhsh A, Vakili S, Moghadam D, Rastegar S, Mohsenizadeh M, Jahromi BN, Vafadar A, Zarei R. Non-apoptotic cell death programs in cervical cancer with an emphasis on ferroptosis. *Crit Rev Oncol Hematol* 2024; **194**: 104249 [PMID: 38145831 DOI: 10.1016/j.critrevonc.2023.104249]

89 **Stefanini B**, Ielasi L, Chen R, Abbati C, Tonnini M, Tovoli F, Granito A. TKIs in combination with immunotherapy for hepatocellular carcinoma. *Expert Rev Anticancer Ther* 2023; **23**: 279-291 [PMID: 36794716 DOI: 10.1080/14737140.2023.2181162]

90 **Nie J**, Lin B, Zhou M, Wu L, Zheng T. Role of ferroptosis in hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2018; **144**: 2329-2337 [PMID: 30167889 DOI: 10.1007/s00432-018-2740-3]

91 **Iseda N**, Itoh S, Toshida K, Tomiyama T, Morinaga A, Shimokawa M, Shimagaki T, Wang H, Kurihara T, Toshima T, Nagao Y, Harada N, Yoshizumi T, Mori M. Ferroptosis is induced by lenvatinib through fibroblast growth factor receptor-4 inhibition in hepatocellular carcinoma. *Cancer Sci* 2022; **113**: 2272-2287 [PMID: 35466502 DOI: 10.1111/cas.15378]

92 **Granito A**, Marinelli S, Terzi E, Piscaglia F, Renzulli M, Venerandi L, Benevento F, Bolondi L. Metronomic capecitabine as second-line treatment in hepatocellular carcinoma after sorafenib failure. *Dig Liver Dis* 2015; **47**: 518-522 [PMID: 25861840 DOI: 10.1016/j.dld.2015.03.010]

93 **Trevisani F**, Brandi G, Garuti F, Barbera MA, Tortora R, Casadei Gardini A, Granito A, Tovoli F, De Lorenzo S, Inghilesi AL, Foschi FG, Bernardi M, Marra F, Sacco R, Di Costanzo GG. Metronomic capecitabine as second-line treatment for hepatocellular carcinoma after sorafenib discontinuation. *J Cancer Res Clin Oncol* 2018; **144**: 403-414 [PMID: 29249005 DOI: 10.1007/s00432-017-2556-6]

94 **Wang H**, Yang R, Wang Z, Cao L, Kong D, Sun Q, Yoshida S, Ren J, Chen T, Duan J, Lu J, Shen Z, Zheng H. Metronomic capecitabine with rapamycin exerts an immunosuppressive effect by inducing ferroptosis of CD4(+) T cells after liver transplantation in rat. *Int Immunopharmacol* 2023; **124**: 110810 [PMID: 37625370 DOI: 10.1016/j.intimp.2023.110810]

95 **Li ZJ**, Dai HQ, Huang XW, Feng J, Deng JH, Wang ZX, Yang XM, Liu YJ, Wu Y, Chen PH, Shi H, Wang JG, Zhou J, Lu GD. Artesunate synergizes with sorafenib to induce ferroptosis in hepatocellular carcinoma. *Acta Pharmacol Sin* 2021; **42**: 301-310 [PMID: 32699265 DOI: 10.1038/s41401-020-0478-3]

96 **Wang Q**, Bin C, Xue Q, Gao Q, Huang A, Wang K, Tang N. GSTZ1 sensitizes hepatocellular carcinoma cells to sorafenib-induced ferroptosis via inhibition of NRF2/GPX4 axis. *Cell Death Dis* 2021; **12**: 426 [PMID: 33931597 DOI: 10.1038/s41419-021-03718-4]

97 **Yang C**, Lu T, Liu M, Yuan X, Li D, Zhang J, Zhou L, Xu M. Tiliroside targets TBK1 to induce ferroptosis and sensitize hepatocellular carcinoma to sorafenib. *Phytomedicine* 2023; **111**: 154668 [PMID: 36657316 DOI: 10.1016/j.phymed.2023.154668]

98 **Ray CA**, Pickup DJ. The mode of death of pig kidney cells infected with cowpox virus is governed by the expression of the crmA gene. *Virology* 1996; **217**: 384-391 [PMID: 8599227 DOI: 10.1006/viro.1996.0128]

99 **Degterev A**, Huang Z, Boyce M, Li Y, Jagtap P, Mizushima N, Cuny GD, Mitchison TJ, Moskowitz MA, Yuan J. Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nat Chem Biol* 2005; **1**: 112-119 [PMID: 16408008 DOI: 10.1038/nchembio711]

100 **He S**, Wang L, Miao L, Wang T, Du F, Zhao L, Wang X. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. *Cell* 2009; **137**: 1100-1111 [PMID: 19524512 DOI: 10.1016/j.cell.2009.05.021]

101 **Zhang DW**, Shao J, Lin J, Zhang N, Lu BJ, Lin SC, Dong MQ, Han J. RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. *Science* 2009; **325**: 332-336 [PMID: 19498109 DOI: 10.1126/science.1172308]

102 **Zhao J**, Jitkaew S, Cai Z, Choksi S, Li Q, Luo J, Liu ZG. Mixed lineage kinase domain-like is a key receptor interacting protein 3 downstream component of TNF-induced necrosis. *Proc Natl Acad Sci U S A* 2012; **109**: 5322-5327 [PMID: 22421439 DOI: 10.1073/pnas.1200012109]

103 **Frank D**, Vince JE. Pyroptosis versus necroptosis: similarities, differences, and crosstalk. *Cell Death Differ* 2019; **26**: 99-114 [PMID: 30341423 DOI: 10.1038/s41418-018-0212-6]

104 **Weinlich R**, Oberst A, Beere HM, Green DR. Necroptosis in development, inflammation and disease. *Nat Rev Mol Cell Biol* 2017; **18**: 127-136 [PMID: 27999438 DOI: 10.1038/nrm.2016.149]

105 **Mompeán M**, Li W, Li J, Laage S, Siemer AB, Bozkurt G, Wu H, McDermott AE. The Structure of the Necrosome RIPK1-RIPK3 Core, a Human Hetero-Amyloid Signaling Complex. *Cell* 2018; **173**: 1244-1253.e10 [PMID: 29681455 DOI: 10.1016/j.cell.2018.03.032]

106 **Hanna-Addams S**, Liu S, Liu H, Chen S, Wang Z. CK1α, CK1δ, and CK1ε are necrosome components which phosphorylate serine 227 of human RIPK3 to activate necroptosis. *Proc Natl Acad Sci U S A* 2020; **117**: 1962-1970 [PMID: 31932442 DOI: 10.1073/pnas.1917112117]

107 **Cai Z**, Jitkaew S, Zhao J, Chiang HC, Choksi S, Liu J, Ward Y, Wu LG, Liu ZG. Plasma membrane translocation of trimerized MLKL protein is required for TNF-induced necroptosis. *Nat Cell Biol* 2014; **16**: 55-65 [PMID: 24316671 DOI: 10.1038/ncb2883]

108 **Cai Z**, Zhang A, Choksi S, Li W, Li T, Zhang XM, Liu ZG. Activation of cell-surface proteases promotes necroptosis, inflammation and cell migration. *Cell Res* 2016; **26**: 886-900 [PMID: 27444869 DOI: 10.1038/cr.2016.87]

109 **Furuta Y**, Zhou Z. How do necrotic cells expose phosphatidylserine to attract their predators-What's unique and what's in common with apoptotic cells. *Front Cell Dev Biol* 2023; **11**: 1170551 [PMID: 37091984 DOI: 10.3389/fcell.2023.1170551]

110 **Gullett JM**, Tweedell RE, Kanneganti TD. It's All in the PAN: Crosstalk, Plasticity, Redundancies, Switches, and Interconnectedness Encompassed by PANoptosis Underlying the Totality of Cell Death-Associated Biological Effects. *Cells* 2022; **11** [PMID: 35563804 DOI: 10.3390/cells11091495]

111 **Li X**, Li F, Zhang X, Zhang H, Zhao Q, Li M, Wu X, Wang L, Liu J, Wu X, Ou Y, Xing M, Zhang Y, Deng J, Wang X, Luo Y, Li J, Zhao Y, Zhang H. Caspase-8 auto-cleavage regulates programmed cell death and collaborates with RIPK3/MLKL to prevent lymphopenia. *Cell Death Differ* 2022; **29**: 1500-1512 [PMID: 35064213 DOI: 10.1038/s41418-022-00938-9]

112 **Contreras CJ**, Mukherjee N, Branco RCS, Lin L, Hogan MF, Cai EP, Oberst AA, Kahn SE, Templin AT. RIPK1 and RIPK3 regulate TNFα-induced β-cell death in concert with caspase activity. *Mol Metab* 2022; **65**: 101582 [PMID: 36030035 DOI: 10.1016/j.molmet.2022.101582]

113 **Chen XY**, Dai YH, Wan XX, Hu XM, Zhao WJ, Ban XX, Wan H, Huang K, Zhang Q, Xiong K. ZBP1-Mediated Necroptosis: Mechanisms and Therapeutic Implications. *Molecules* 2022; **28** [PMID: 36615244 DOI: 10.3390/molecules28010052]

114 **Nakano H**, Murai S, Moriwaki K. Regulation of the release of damage-associated molecular patterns from necroptotic cells. *Biochem J* 2022; **479**: 677-685 [PMID: 35293986 DOI: 10.1042/BCJ20210604]

115 **Schock SN**, Chandra NV, Sun Y, Irie T, Kitagawa Y, Gotoh B, Coscoy L, Winoto A. Induction of necroptotic cell death by viral activation of the RIG-I or STING pathway. *Cell Death Differ* 2017; **24**: 615-625 [PMID: 28060376 DOI: 10.1038/cdd.2016.153]

116 **Ye K**, Chen Z, Xu Y. The double-edged functions of necroptosis. *Cell Death Dis* 2023; **14**: 163 [PMID: 36849530 DOI: 10.1038/s41419-023-05691-6]

117 **Wang X**, He Z, Liu H, Yousefi S, Simon HU. Neutrophil Necroptosis Is Triggered by Ligation of Adhesion Molecules following GM-CSF Priming. *J Immunol* 2016; **197**: 4090-4100 [PMID: 27815445 DOI: 10.4049/jimmunol.1600051]

118 **Gong YN**, Guy C, Olauson H, Becker JU, Yang M, Fitzgerald P, Linkermann A, Green DR. ESCRT-III Acts Downstream of MLKL to Regulate Necroptotic Cell Death and Its Consequences. *Cell* 2017; **169**: 286-300.e16 [PMID: 28388412 DOI: 10.1016/j.cell.2017.03.020]

119 **Park SY**, Park HH, Park SY, Hong SM, Yoon S, Morgan MJ, Kim YS. Reduction in MLKL-mediated endosomal trafficking enhances the TRAIL-DR4/5 signal to increase cancer cell death. *Cell Death Dis* 2020; **11**: 744 [PMID: 32917855 DOI: 10.1038/s41419-020-02941-9]

120 **Chavoshinezhad S**, Beirami E, Izadpanah E, Feligioni M, Hassanzadeh K. Molecular mechanism and potential therapeutic targets of necroptosis and ferroptosis in Alzheimer's disease. *Biomed Pharmacother* 2023; **168**: 115656 [PMID: 37844354 DOI: 10.1016/j.biopha.2023.115656]

121 **Kolbrink B**, von Samson-Himmelstjerna FA, Murphy JM, Krautwald S. Role of necroptosis in kidney health and disease. *Nat Rev Nephrol* 2023; **19**: 300-314 [PMID: 36596919 DOI: 10.1038/s41581-022-00658-w]

122 **Yan J**, Wan P, Choksi S, Liu ZG. Necroptosis and tumor progression. *Trends Cancer* 2022; **8**: 21-27 [PMID: 34627742 DOI: 10.1016/j.trecan.2021.09.003]

123 **Aravinthan A**, Scarpini C, Tachtatzis P, Verma S, Penrhyn-Lowe S, Harvey R, Davies SE, Allison M, Coleman N, Alexander G. Hepatocyte senescence predicts progression in non-alcohol-related fatty liver disease. *J Hepatol* 2013; **58**: 549-556 [PMID: 23142622 DOI: 10.1016/j.jhep.2012.10.031]

124 **Carranza-Trejo AM**, Vetvicka V, Vistejnova L, Kralickova M, Montufar EB. Hepatocyte and immune cell crosstalk in non-alcoholic fatty liver disease. *Expert Rev Gastroenterol Hepatol* 2021; **15**: 783-796 [PMID: 33557653 DOI: 10.1080/17474124.2021.1887730]

125 **Vanni E**, Bugianesi E, Kotronen A, De Minicis S, Yki-Järvinen H, Svegliati-Baroni G. From the metabolic syndrome to NAFLD or vice versa? *Dig Liver Dis* 2010; **42**: 320-330 [PMID: 20207596 DOI: 10.1016/j.dld.2010.01.016]

126 **Agmon E**, Stockwell BR. Lipid homeostasis and regulated cell death. *Curr Opin Chem Biol* 2017; **39**: 83-89 [PMID: 28645028 DOI: 10.1016/j.cbpa.2017.06.002]

127 **Newton K**, Manning G. Necroptosis and Inflammation. *Annu Rev Biochem* 2016; **85**: 743-763 [PMID: 26865533 DOI: 10.1146/annurev-biochem-060815-014830]

128 **Islam T**, Afonso MB, Rodrigues CMP. The role of RIPK3 in liver mitochondria bioenergetics and function. *Eur J Clin Invest* 2022; **52**: e13648 [PMID: 34219227 DOI: 10.1111/eci.13648]

129 **Leven AS**, Gieseler RK, Schlattjan M, Schreiter T, Niedergethmann M, Baars T, Baba HA, Özçürümez MK, Sowa JP, Canbay A. Association of cell death mechanisms and fibrosis in visceral white adipose tissue with pathological alterations in the liver of morbidly obese patients with NAFLD. *Adipocyte* 2021; **10**: 558-573 [PMID: 34743657 DOI: 10.1080/21623945.2021.1982164]

130 **Scavo MP**, Negro R, Arrè V, Depalo N, Carrieri L, Rizzi F, Mastrogiacomo R, Serino G, Notarnicola M, De Nunzio V, Lippolis T, Pesole PL, Coletta S, Armentano R, Curri ML, Giannelli G. The oleic/palmitic acid imbalance in exosomes isolated from NAFLD patients induces necroptosis of liver cells via the elongase-6/RIP-1 pathway. *Cell Death Dis* 2023; **14**: 635 [PMID: 37752143 DOI: 10.1038/s41419-023-06161-9]

131 **Seo YY**, Cho YK, Bae JC, Seo MH, Park SE, Rhee EJ, Park CY, Oh KW, Park SW, Lee WY. Tumor Necrosis Factor-α as a Predictor for the Development of Nonalcoholic Fatty Liver Disease: A 4-Year Follow-Up Study. *Endocrinol Metab (Seoul)* 2013; **28**: 41-45 [PMID: 24396649 DOI: 10.3803/EnM.2013.28.1.41]

132 **Zhang W**, Kudo H, Kawai K, Fujisaka S, Usui I, Sugiyama T, Tsukada K, Chen N, Takahara T. Tumor necrosis factor-alpha accelerates apoptosis of steatotic hepatocytes from a murine model of non-alcoholic fatty liver disease. *Biochem Biophys Res Commun* 2010; **391**: 1731-1736 [PMID: 20043871 DOI: 10.1016/j.bbrc.2009.12.144]

133 **Qian LL**, Ji JJ, Jiang Y, Guo JQ, Wu Y, Yang Z, Ma GS, Yao YY. Serpina3c deficiency induced necroptosis promotes non-alcoholic fatty liver disease through β-catenin/Foxo1/TLR4 signaling. *FASEB J* 2022; **36**: e22316 [PMID: 35429042 DOI: 10.1096/fj.202101345RRR]

134 **Coulon S**, Francque S, Colle I, Verrijken A, Blomme B, Heindryckx F, De Munter S, Prawitt J, Caron S, Staels B, Van Vlierberghe H, Van Gaal L, Geerts A. Evaluation of inflammatory and angiogenic factors in patients with non-alcoholic fatty liver disease. *Cytokine* 2012; **59**: 442-449 [PMID: 22658783 DOI: 10.1016/j.cyto.2012.05.001]

135 **Xinyu W**, Qian W, Yanjun W, Jingwen K, Keying X, Jiazheng J, Haibing Z, Kai W, Xiao X, Lixing Z. Polarity protein AF6 functions as a modulator of necroptosis by regulating ubiquitination of RIPK1 in liver diseases. *Cell Death Dis* 2023; **14**: 673 [PMID: 37828052 DOI: 10.1038/s41419-023-06170-8]

136 **Preston SP**, Stutz MD, Allison CC, Nachbur U, Gouil Q, Tran BM, Duvivier V, Arandjelovic P, Cooney JP, Mackiewicz L, Meng Y, Schaefer J, Bader SM, Peng H, Valaydon Z, Rajasekaran P, Jennison C, Lopaticki S, Farrell A, Ryan M, Howell J, Croagh C, Karunakaran D, Schuster-Klein C, Murphy JM, Fifis T, Christophi C, Vincan E, Blewitt ME, Thompson A, Boddey JA, Doerflinger M, Pellegrini M. Epigenetic Silencing of RIPK3 in Hepatocytes Prevents MLKL-mediated Necroptosis From Contributing to Liver Pathologies. *Gastroenterology* 2022; **163**: 1643-1657.e14 [PMID: 36037995 DOI: 10.1053/j.gastro.2022.08.040]

137 **Wu X**, Poulsen KL, Sanz-Garcia C, Huang E, McMullen MR, Roychowdhury S, Dasarathy S, Nagy LE. MLKL-dependent signaling regulates autophagic flux in a murine model of non-alcohol-associated fatty liver and steatohepatitis. *J Hepatol* 2020; **73**: 616-627 [PMID: 32220583 DOI: 10.1016/j.jhep.2020.03.023]

138 **Ding HR**, Tang ZT, Tang N, Zhu ZY, Liu HY, Pan CY, Hu AY, Lin YZ, Gou P, Yuan XW, Cai JH, Dong CL, Wang JL, Ren HZ. Protective Properties of FOXO1 Inhibition in a Murine Model of Non-alcoholic Fatty Liver Disease Are Associated With Attenuation of ER Stress and Necroptosis. *Front Physiol* 2020; **11**: 177 [PMID: 32218743 DOI: 10.3389/fphys.2020.00177]

139 **Mohammed S**, Nicklas EH, Thadathil N, Selvarani R, Royce GH, Kinter M, Richardson A, Deepa SS. Role of necroptosis in chronic hepatic inflammation and fibrosis in a mouse model of increased oxidative stress. *Free Radic Biol Med* 2021; **164**: 315-328 [PMID: 33429022 DOI: 10.1016/j.freeradbiomed.2020.12.449]

140 **Oh JH**, Saeed WK, Kim HY, Lee SM, Lee AH, Park GR, Yoon EL, Jun DW. Hepatic stellate cells activate and avoid death under necroptosis stimuli: Hepatic fibrosis during necroptosis. *J Gastroenterol Hepatol* 2023; **38**: 2206-2214 [PMID: 37811601 DOI: 10.1111/jgh.16368]

141 **Inaba Y**, Hashiuchi E, Watanabe H, Kimura K, Oshima Y, Tsuchiya K, Murai S, Takahashi C, Matsumoto M, Kitajima S, Yamamoto Y, Honda M, Asahara SI, Ravnskjaer K, Horike SI, Kaneko S, Kasuga M, Nakano H, Harada K, Inoue H. The transcription factor ATF3 switches cell death from apoptosis to necroptosis in hepatic steatosis in male mice. *Nat Commun* 2023; **14**: 167 [PMID: 36690638 DOI: 10.1038/s41467-023-35804-w]

142 **Zhang NP**, Liu XJ, Xie L, Shen XZ, Wu J. Impaired mitophagy triggers NLRP3 inflammasome activation during the progression from nonalcoholic fatty liver to nonalcoholic steatohepatitis. *Lab Invest* 2019; **99**: 749-763 [PMID: 30700851 DOI: 10.1038/s41374-018-0177-6]

143 **Mridha AR**, Haczeyni F, Yeh MM, Haigh WG, Ioannou GN, Barn V, Ajamieh H, Adams L, Hamdorf JM, Teoh NC, Farrell GC. TLR9 is up-regulated in human and murine NASH: pivotal role in inflammatory recruitment and cell survival. *Clin Sci (Lond)* 2017; **131**: 2145-2159 [PMID: 28687713 DOI: 10.1042/CS20160838]

144 **Tomita K**, Tamiya G, Ando S, Ohsumi K, Chiyo T, Mizutani A, Kitamura N, Toda K, Kaneko T, Horie Y, Han JY, Kato S, Shimoda M, Oike Y, Tomizawa M, Makino S, Ohkura T, Saito H, Kumagai N, Nagata H, Ishii H, Hibi T. Tumour necrosis factor alpha signalling through activation of Kupffer cells plays an essential role in liver fibrosis of non-alcoholic steatohepatitis in mice. *Gut* 2006; **55**: 415-424 [PMID: 16174657 DOI: 10.1136/gut.2005.071118]

145 **Afonso MB**, Rodrigues PM, Carvalho T, Caridade M, Borralho P, Cortez-Pinto H, Castro RE, Rodrigues CM. Necroptosis is a key pathogenic event in human and experimental murine models of non-alcoholic steatohepatitis. *Clin Sci (Lond)* 2015; **129**: 721-739 [PMID: 26201023 DOI: 10.1042/CS20140732]

146 **Gautheron J**, Vucur M, Reisinger F, Cardenas DV, Roderburg C, Koppe C, Kreggenwinkel K, Schneider AT, Bartneck M, Neumann UP, Canbay A, Reeves HL, Luedde M, Tacke F, Trautwein C, Heikenwalder M, Luedde T. A positive feedback loop between RIP3 and JNK controls non-alcoholic steatohepatitis. *EMBO Mol Med* 2014; **6**: 1062-1074 [PMID: 24963148 DOI: 10.15252/emmm.201403856]

147 **Tao L**, Yi Y, Chen Y, Zhang H, Orning P, Lien E, Jie J, Zhang W, Xu Q, Li Y, Ding Z, Wu C, Ding Q, Wang J, Zhang J, Weng D. RIP1 kinase activity promotes steatohepatitis through mediating cell death and inflammation in macrophages. *Cell Death Differ* 2021; **28**: 1418-1433 [PMID: 33208891 DOI: 10.1038/s41418-020-00668-w]

148 **Liu XJ**, Duan NN, Liu C, Niu C, Liu XP, Wu J. Characterization of a murine nonalcoholic steatohepatitis model induced by high fat high calorie diet plus fructose and glucose in drinking water. *Lab Invest* 2018; **98**: 1184-1199 [PMID: 29959418 DOI: 10.1038/s41374-018-0074-z]

149 **Saeed WK**, Jun DW, Jang K, Ahn SB, Oh JH, Chae YJ, Lee JS, Kang HT. Mismatched effects of receptor interacting protein kinase-3 on hepatic steatosis and inflammation in non-alcoholic fatty liver disease. *World J Gastroenterol* 2018; **24**: 5477-5490 [PMID: 30622377 DOI: 10.3748/wjg.v24.i48.5477]

150 **Afonso MB**, Rodrigues PM, Mateus-Pinheiro M, Simão AL, Gaspar MM, Majdi A, Arretxe E, Alonso C, Santos-Laso A, Jimenez-Agüero R, Eizaguirre E, Bujanda L, Pareja MJ, Banales JM, Ratziu V, Gautheron J, Castro RE, Rodrigues CMP. RIPK3 acts as a lipid metabolism regulator contributing to inflammation and carcinogenesis in non-alcoholic fatty liver disease. *Gut* 2021; **70**: 2359-2372 [PMID: 33361348 DOI: 10.1136/gutjnl-2020-321767]

151 **Mohammed S**, Thadathil N, Ohene-Marfo P, Tran AL, Van Der Veldt M, Georgescu C, Oh S, Nicklas EH, Wang D, Haritha NH, Luo W, Janknecht R, Miller BF, Wren JD, Freeman WM, Deepa SS. Absence of Either Ripk3 or Mlkl Reduces Incidence of Hepatocellular Carcinoma Independent of Liver Fibrosis. *Mol Cancer Res* 2023; **21**: 933-946 [PMID: 37204757 DOI: 10.1158/1541-7786.MCR-22-0820]

152 **Wu W**, Hu X, Zhou X, Klenotic PA, Zhou Q, Lin Z. Myeloid deficiency of CCN3 exacerbates liver injury in a mouse model of nonalcoholic fatty liver disease. *J Cell Commun Signal* 2018; **12**: 389-399 [PMID: 29214510 DOI: 10.1007/s12079-017-0432-4]

153 **Chen H**, McKeen T, Chao X, Chen A, Deng F, Jaeschke H, Ding WX, Ni HM. The role of MLKL in Hepatic Ischemia-Reperfusion Injury of Alcoholic Steatotic Livers. *Int J Biol Sci* 2022; **18**: 1096-1106 [PMID: 35173541 DOI: 10.7150/ijbs.67533]

154 **Yang F**, Shang L, Wang S, Liu Y, Ren H, Zhu W, Shi X. TNFα-Mediated Necroptosis Aggravates Ischemia-Reperfusion Injury in the Fatty Liver by Regulating the Inflammatory Response. *Oxid Med Cell Longev* 2019; **2019**: 2301903 [PMID: 31214277 DOI: 10.1155/2019/2301903]

155 **Mazzolini G**, Atorrasagasti C, Onorato A, Peixoto E, Schlattjan M, Sowa JP, Sydor S, Gerken G, Canbay A. SPARC expression is associated with hepatic injury in rodents and humans with non-alcoholic fatty liver disease. *Sci Rep* 2018; **8**: 725 [PMID: 29335425 DOI: 10.1038/s41598-017-18981-9]

156 **Paredes-Turrubiarte G**, González-Chávez A, Pérez-Tamayo R, Salazar-Vázquez BY, Hernández VS, Garibay-Nieto N, Fragoso JM, Escobedo G. Severity of non-alcoholic fatty liver disease is associated with high systemic levels of tumor necrosis factor alpha and low serum interleukin 10 in morbidly obese patients. *Clin Exp Med* 2016; **16**: 193-202 [PMID: 25894568 DOI: 10.1007/s10238-015-0347-4]

157 **Poniachik J**, Csendes A, Díaz JC, Rojas J, Burdiles P, Maluenda F, Smok G, Rodrigo R, Videla LA. Increased production of IL-1alpha and TNF-alpha in lipopolysaccharide-stimulated blood from obese patients with non-alcoholic fatty liver disease. *Cytokine* 2006; **33**: 252-257 [PMID: 16564703 DOI: 10.1016/j.cyto.2006.02.006]

158 **Zahran WE**, Salah El-Dien KA, Kamel PG, El-Sawaby AS. Efficacy of Tumor Necrosis Factor and Interleukin-10 Analysis in the Follow-up of Nonalcoholic Fatty Liver Disease Progression. *Indian J Clin Biochem* 2013; **28**: 141-146 [PMID: 24426199 DOI: 10.1007/s12291-012-0236-5]

159 **Cheng Y**, An B, Jiang M, Xin Y, Xuan S. Association of Tumor Necrosis Factor-alpha Polymorphisms and Risk of Coronary Artery Disease in Patients With Non-alcoholic Fatty Liver Disease. *Hepat Mon* 2015; **15**: e26818 [PMID: 25825591 DOI: 10.5812/hepatmon.26818]

160 **Miyata T**, Wu X, Fan X, Huang E, Sanz-Garcia C, Ross CKC, Roychowdhury S, Bellar A, McMullen MR, Dasarathy J, Allende DS, Caballeria J, Sancho-Bru P, McClain CJ, Mitchell M, McCullough AJ, Radaeva S, Barton B, Szabo G, Dasarathy S, Nagy LE. Differential role of MLKL in alcohol-associated and non-alcohol-associated fatty liver diseases in mice and humans. *JCI Insight* 2021; **6** [PMID: 33616081 DOI: 10.1172/jci.insight.140180]

161 **Kondo T**, Macdonald S, Engelmann C, Habtesion A, Macnaughtan J, Mehta G, Mookerjee RP, Davies N, Pavesi M, Moreau R, Angeli P, Arroyo V, Andreola F, Jalan R. The role of RIPK1 mediated cell death in acute on chronic liver failure. *Cell Death Dis* 2021; **13**: 5 [PMID: 34921136 DOI: 10.1038/s41419-021-04442-9]

162 **Ahmed EA**, El-Derany MO, Anwar AM, Saied EM, Magdeldin S. Metabolomics and Lipidomics Screening Reveal Reprogrammed Signaling Pathways toward Cancer Development in Non-Alcoholic Steatohepatitis. *Int J Mol Sci* 2022; **24** [PMID: 36613653 DOI: 10.3390/ijms24010210]

163 **Majdi A**, Aoudjehane L, Ratziu V, Islam T, Afonso MB, Conti F, Mestiri T, Lagouge M, Foufelle F, Ballenghien F, Ledent T, Moldes M, Cadoret A, Fouassier L, Delaunay JL, Aït-Slimane T, Courtois G, Fève B, Scatton O, Prip-Buus C, Rodrigues CMP, Housset C, Gautheron J. Inhibition of receptor-interacting protein kinase 1 improves experimental non-alcoholic fatty liver disease. *J Hepatol* 2020; **72**: 627-635 [PMID: 31760070 DOI: 10.1016/j.jhep.2019.11.008]

164 **Saeed WK**, Jun DW, Jang K, Oh JH, Chae YJ, Lee JS, Koh DH, Kang HT. Decrease in fat de novo synthesis and chemokine ligand expression in non-alcoholic fatty liver disease caused by inhibition of mixed lineage kinase domain-like pseudokinase. *J Gastroenterol Hepatol* 2019; **34**: 2206-2218 [PMID: 31132314 DOI: 10.1111/jgh.14740]

165 **Briand F**, Heymes C, Bonada L, Angles T, Charpentier J, Branchereau M, Brousseau E, Quinsat M, Fazilleau N, Burcelin R, Sulpice T. A 3-week nonalcoholic steatohepatitis mouse model shows elafibranor benefits on hepatic inflammation and cell death. *Clin Transl Sci* 2020; **13**: 529-538 [PMID: 31981449 DOI: 10.1111/cts.12735]

166 **Hua X**, Sun DY, Zhang WJ, Fu JT, Tong J, Sun SJ, Zeng FY, Ouyang SX, Zhang GY, Wang SN, Li DJ, Miao CY, Wang P. P7C3-A20 alleviates fatty liver by shaping gut microbiota and inducing FGF21/FGF1, via the AMP-activated protein kinase/CREB regulated transcription coactivator 2 pathway. *Br J Pharmacol* 2021; **178**: 2111-2130 [PMID: 32037512 DOI: 10.1111/bph.15008]

167 **Malaguarnera L**, Di Rosa M, Zambito AM, dell'Ombra N, Nicoletti F, Malaguarnera M. Chitotriosidase gene expression in Kupffer cells from patients with non-alcoholic fatty liver disease. *Gut* 2006; **55**: 1313-1320 [PMID: 16825325 DOI: 10.1136/gut.2005.075697]

168 **Shao Y**, Wang X, Zhou Y, Jiang Y, Wu R, Lu C. Pterostilbene attenuates RIPK3-dependent hepatocyte necroptosis in alcoholic liver disease via SIRT2-mediated NFATc4 deacetylation. *Toxicology* 2021; **461**: 152923 [PMID: 34474091 DOI: 10.1016/j.tox.2021.152923]

169 **Roychowdhury S**, McCullough RL, Sanz-Garcia C, Saikia P, Alkhouri N, Matloob A, Pollard KA, McMullen MR, Croniger CM, Nagy LE. Receptor interacting protein 3 protects mice from high-fat diet-induced liver injury. *Hepatology* 2016; **64**: 1518-1533 [PMID: 27301788 DOI: 10.1002/hep.28676]

170 **Abdel-Hamed AR**, Hamouda AO, Abo-elmatty DM, Khedr NF, Ghattas MH. Role of Kaempferol Combined with Pioglitazone in the Alleviation of Inflammation and Modulation of Necroptosis and Apoptosis Pathways in NASH-induced Mice. *J Med Chem Sci* 2023; **6**: 250-268 [DOI: 10.26655/JMCHEMSCI.2023.2.8]

171 **Fawzy MA**, Nasr G, Ali FEM, Fathy M. Quercetin potentiates the hepatoprotective effect of sildenafil and/or pentoxifylline against intrahepatic cholestasis: Role of Nrf2/ARE, TLR4/NF-κB, and NLRP3/IL-1β signaling pathways. *Life Sci* 2023; **314**: 121343 [PMID: 36592787 DOI: 10.1016/j.lfs.2022.121343]

172 **Hamouda AO**, Abdel-Hamed AR, Abo-Elmatty DM, Khedr NF, Ghattas MH. Pentoxifylline and its association with kaempferol improve NASH-associated manifestation in mice through anti-apoptotic, anti-necroptotic, antioxidant, and anti-inflammatory mechanisms. *Eur Rev Med Pharmacol Sci* 2022; **26**: 8644-8659 [PMID: 36524484 DOI: 10.26355/eurrev\_202212\_30535]

173 **Park J**, Rah SY, An HS, Lee JY, Roh GS, Ryter SW, Park JW, Yang CH, Surh YJ, Kim UH, Chung HT, Joe Y. Metformin-induced TTP mediates communication between Kupffer cells and hepatocytes to alleviate hepatic steatosis by regulating lipophagy and necroptosis. *Metabolism* 2023; **141**: 155516 [PMID: 36773805 DOI: 10.1016/j.metabol.2023.155516]

174 **Xiao J**, Ho CT, Liong EC, Nanji AA, Leung TM, Lau TY, Fung ML, Tipoe GL. Epigallocatechin gallate attenuates fibrosis, oxidative stress, and inflammation in non-alcoholic fatty liver disease rat model through TGF/SMAD, PI3 K/Akt/FoxO1, and NF-kappa B pathways. *Eur J Nutr* 2014; **53**: 187-199 [PMID: 23515587 DOI: 10.1007/s00394-013-0516-8]

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**Table 1 The unique morphological and biochemical hallmarks of regulated cell deaths**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Morphological characteristics** | **Biochemical hallmarks** |
| Apoptotic RCD | Apoptosis | Cell and nucleus shrinkage, nuclear chromatin condensation, karyorrhexis, the formation of apoptotic bodies, and cell fragmentation | ↑CASPs (8, 9, 3, and 7), ↑BAD and BAX, ↓BCL2 |
| Non-apoptotic RCDs | Pyroptosis | Inflammasomes caused membrane rupture, cell swelling/cell lysis, DNA fragmentation, nuclear condensation, and nuclear pores | ↑CASPs (1 and 7), ↑GSDMs (D, E, *etc.*), ↑IL-18, ↑IL-1β |
| Ferroptosis | Cell swelling, mitochondria shrinkage, cristae disappearance, increased density of mitochondrial membrane, the rupture of the outer mitochondrial membrane | ↑Fe2+, ROS, lipid peroxidation, ↑ACSL4 and PTGS2, ↓GPX4 and GSH |
| Necroptosis | Organelles swelling, cell membrane rupture, cell lysis, loss of cell membrane integrity, nuclear chromatin deficiency | ↑RIPK1, ↑RIPK3, ↑MLKL, ↓CASP-8 |

RIPK1: Receptor-interacting protein kinase 1; MLKL: Mixed lineage kinase domain-like pseudokinase; RCD: Regulated cell death; CASP: Caspase; ROS: Reactive oxygen species; GSDM: Gasdermin; IL: Interleukin; ACSL4: Acyl-CoA synthetase long-chain family member 4; GPX4: Glutathione peroxidase 4; GSH: Glutathione.

**Table 2 Participation of necroptosis in the occurrence and progression of the disease and its effectiveness as a diagnostic marker and therapeutic target**

|  |  |
| --- | --- |
| **Function** | **Advantages/limitations** |
| Pathoetiology | The upregulation of RIPK1, RIPK3, and MLKL leads to the necroptosis of hepatocytes in the fatty liver, which along with inflammatory responses contributes to the induction of NAFLD. In addition, the intensification of necroptotic death of hepatocytes is followed by the aggravation of the disease and its progress toward NASH and HCC |
| Diagnosis | Most of the presented biomarkers are related to the upstream inflammatory inducers of necroptosis such as TNF and ILs. Although the aforementioned markers have provided diagnostic and prognostic properties, changes in inflammatory markers occur in a variety of disorders. In addition, the uncertainty of the sensitivity and specificity of the few suggested markers complicates the evaluation of their diagnostic value. Therefore, more studies that evaluate specific necroptotic biomarkers in NAFLD patients are encouraged |
| Therapy | Direct inhibitors of necroptosis (RIPK1 inhibitors for example) and a variety of herbal antioxidants with anti-necroptotic, anti-inflammatory, and regulating lipid metabolism properties have been proposed in experimental and human studies. However, no clinical trial has been registered in this direction, which reveals the necessity of designing further studies |

NAFLD: Nonalcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; HCC: Hepatocellular carcinoma; TNF: Tumor necrosis factor; IL: Interleukin; RIPK1: Receptor-interacting protein kinase 1; MLKL: Mixed lineage kinase domain-like pseudokinase.