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REVIEW

Death Receptor-Mediated Cell Death and Proinflammatory Signaling in Nonalcoholic Steatohepatitis

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SUMMARY

This review discusses recent developments in our understanding of hepatocyte death receptor signaling and its mechanistic link to inflammation in steatohepatitis. Tumor necrosis factor-related apoptosis-inducing ligand receptor activation in hepatocytes during lipotoxicity induces release of proinflammatory extracellular vesicles, which, in turn, promote proinflammatory macrophage activation.

Nonalcoholic fatty liver disease (NAFLD) is becoming a public health problem worldwide. A subset of patients develop an inflammatory disease, nonalcoholic steatohepatitis (NASH), characterized by steatosis, hepatocellular death, macrophage and neutrophil accumulation, and varying stages of fibrosis. Hepatocyte cell death triggers the cellular inflammatory response, therefore reducing cell death may be salutary in the steatohepatitis disease process. Recently, a better understanding of hepatocyte apoptosis in NASH has been obtained and new information regarding other cell death modes such as necroptosis and pyroptosis has been reported. Hepatocyte lipotoxicity is often triggered by death receptors. In addition to causing apoptosis, death receptors have been shown to mediate proinflammatory signaling, suggesting that apoptosis in this context is not an immunologically silent process. Here, we review recent developments in our understanding of hepatocyte cell death by death receptors and its mechanistic link to inflammation in NASH. We emphasize how proapoptotic signaling by death receptors may induce the release of proinflammatory extracellular vesicles, thereby recruiting and activating macrophages and promoting the steatohepatitis process. Potential therapeutic strategies are discussed based on this evolving information. ***(Cell Mol Gastroenterol Hepatol 2015;1:17–27; http://dx.doi.org/10.1016/j.jcmgh.2014.11.005)***

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Nonalcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease in the United States and other Western countries. It is estimated that up to 30% of the Western world's population is affected by NAFLD.[1] A subset of patients with NAFLD (~10% to 25%) develop nonalcoholic steatohepatitis (NASH), a more severe form of the disease characterized by hepatocellular death, accumulation of inflammatory cells, and varying stages of fibrosis. NASH can further progress to end-stage liver disease, cirrhosis, and hepatocellular carcinoma. NASH has become a significant public health concern, confounded by the lack of effective therapies. Thus, there is an urgent and unmet need for effective treatment that would parallel patients' lifestyle modifications. Insight regarding the molecular and cellular mechanisms underlying disease development and progression may provide the impetus for rationale therapeutic strategies.

Cell death is a cardinal feature of NASH, as is accumulation of inflammatory cells, especially macrophages and neutrophils. Although it is widely accepted that cell death promotes cellular inflammation, the mechanistic link between cell death and hepatic inflammation remains enigmatic. Recent information links death receptor signaling to cell death in NASH by processes that promote cell-based inflammation. Thus, it is both timely and topical to review this information and emphasize therapeutic opportunities.

Modes of Cell Death

Hepatocyte apoptosis is the predominant cell death pathway in NASH. In addition, other types of programmed

Abbreviations used in this paper: cFLIP, cellular FLICE/caspase 8-like inhibitory protein; ER, endoplasmic reticulum; FFA, free fatty acid; FADD, Fas-associated protein with death domain; FasL, Fas ligand; fasudil, 5-(1,4-diazepan-1-ylsulfonyl)isoquinoline; GS-9450, (5*R*)-*N*-[(2*S*,3*S*)-2-(fluoromethyl)-2-hydroxy-5-oxooxolan-3-yl]-3-isoquinolin-8-yl-5-propan-2-yl-4*H*-1,2-oxazole-5-carboxamide; HSC, hepatic stellate cells; IDN-6556, (3S)-3-[[(2S)-2-[[2-(2-tert-butylanilino)-2-oxoacetyl]amino]propanoyl]amino]-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoic acid; IL, interleukin; JNK, c-Jun N-terminal kinase; MCD, methionine/choline-deficient; MCP-1, monocyte chemotactic protein-1; MLKL, mixed lineage kinase domain-like protein; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NF-κB, nuclear factor kappa B; NK, natural killer; NKT, natural killer T; NLRP3, NLR family, pyrin domain containing 3; PUMA, p53 up-regulated modulator of apoptosis; RIP, receptor-interacting protein kinase; ROCK1, Rho-associated, coiled-coil containing protein kinase 1; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TRAIL-R, tumor necrosis factor-related apoptosis-inducing ligand receptor; VX-166, (3S)-3-[[(2S)-2-[3-(methoxycarbonylamino)-2-oxopyridin-1-yl]butanoyl]amino]-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoic acid; VX-740, (4*S*,7*S*)-*N*-[(2*R*,3*S*)-2-ethoxy-5-oxooxolan-3-yl]-7-(isoquinoline-1-carbonylamino)-6,10-dioxo-2,3,4,7,8,9-hexahydro-1*H*-pyridazino[1,2-*a*]diazepine-4-carboxamide; VX-765, (2*S*)-1-[(2*S*)-2-[(4-amino-3-chlorobenzoyl)amino]-3,3-dimethylbutanoyl]-*N*-[(2*R*)-2-ethoxy-5-oxooxolan-3-yl]pyrrolidine-2-carboxamide.

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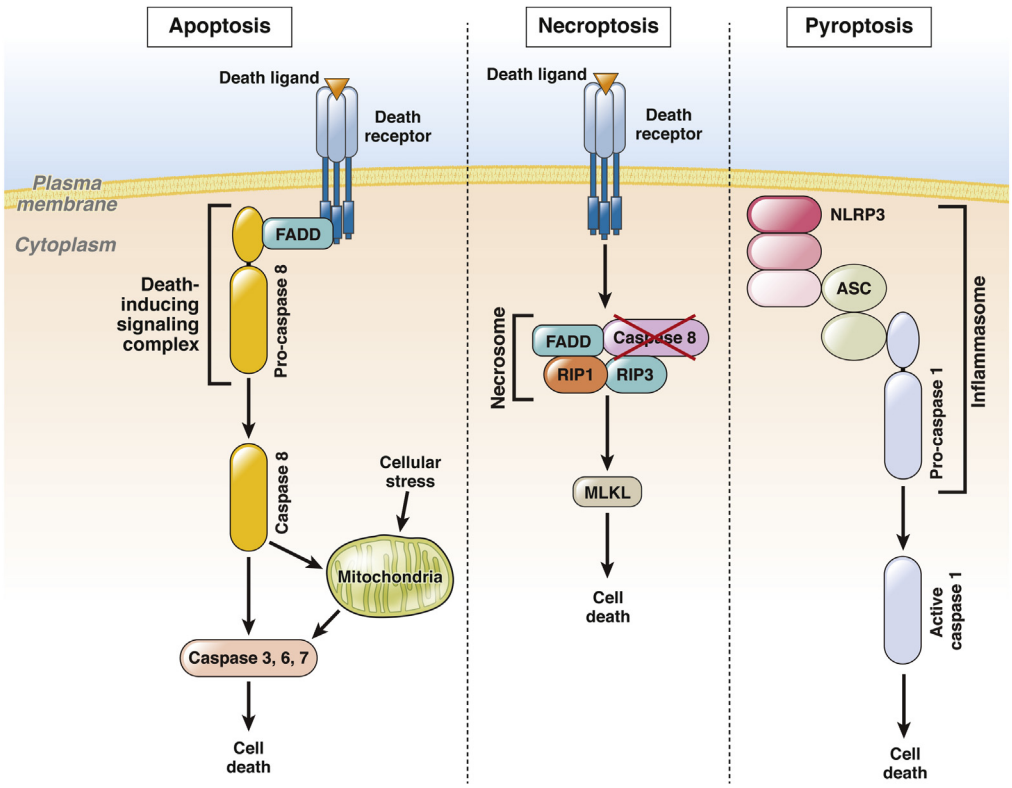

Figure 1. Modes of cell death. Activation of apoptotic, necroptotic, or pyroptotic signaling pathways leads to cell death. *Left panel*: Upon binding of a death ligand, the intracellular domain of a death receptor recruits adaptor proteins such as Fas-associated protein with death domain (FADD) and pro-caspase 8 to form a signaling platform termed the death-inducing signaling complex (DISC). Caspase 8 undergoes proteolytic autoactivation, resulting in direct or indirect (via mitochondria) activation of caspases 3, 6, and 7, which execute the final step of cell death. Various stimuli, including endoplasmic reticulum stress, trigger apoptosis via the intrinsic pathway, where mitochondrial permeabilization is a central event. Release of proapoptotic factors from mitochondria into the cytosol activates caspase 3, 6, and 7 and cell death by apoptosis. *Middle panel*: Necroptosis is initiated by death receptors in cells where caspase 8 function is inhibited. Under these conditions, death receptor activation promotes formation of a necrosome, a signaling complex comprising FADD, receptor-interacting protein kinase 1 (RIP1), and RIP3. This complex induces cell death via mixed lineage kinase domain-like protein (MLKL), and probably other mediators. *Right panel*: Pyroptosis is a cell death mode resulting from activation of inflammasomes, including NLRP3 inflammasome. Upon activation, the intracellular receptor NLR family, pyrin domain containing 3 (NLRP3) recruits apoptosis-associated speck-like protein containing a CARD (ASC) and pro-caspase 1. Cleavage-activated caspase 1 then induces cell death.

cell death such as necroptosis and pyroptosis have been reported. These cell death modes remain largely unexplored and merit further examination. Cell death modes such as necrosis and autophagy are not discussed in this review as they are not triggered by death receptors. These cell biological processes and their respective roles in liver pathobiology and steatohepatitis are reviewed elsewhere.[2,3]

Apoptosis

Apoptosis is highly organized cell death process that is morphologically characterized by cell shrinkage, membrane blebbing, chromatin condensation, DNA fragmentation, and formation of apoptotic bodies. Importantly, apoptotic cell death depends on the activity of caspases, a family of cysteine-dependent aspartate-specific proteases. Apoptosis can be initiated by two fundamental pathways: extrinsic and intrinsic pathways. The extrinsic pathway is initiated by death receptors that belong to tumor necrosis factor (TNF) receptor superfamily. Hepatocytes express death receptors Fas, TNF receptor 1 (TNFR1), and TNF-related apoptosis-inducing ligand (TRAIL) receptor (TRAIL-R) 1 and 2. Activation of these receptors leads to formation of a death-inducing signaling complex (DISC), which consists of caspase 8 and adaptor proteins, such as Fas-associated protein with death domain (FADD, Figure 1). In hepatocytes, activated caspase 8 cleaves Bid, generating tBid, which then induces the cytosolic egress of proapoptotic factors from the intermitochondrial space (eg, cytochrome c). Downstream of mitochondrial dysfunction, cytochrome c promotes formation of the apoptosome, which activates caspase 3, 6, and 7. These effector caspases execute cellular demolition. In hepatocytes, apoptosis is often initiated by the death receptor pathway.[4]

The intrinsic pathway of apoptosis is mediated by mitochondrial or lysosomal permeabilization. In addition, signaling between the endoplasmic reticulum (ER) and mitochondria can promote apoptosis. The intrinsic pathway of