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Hepatocyte Apoptosis at the Interplay of Intracellular Organelles and Membrane-Bound Receptors: Targets for Therapy

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Abstract

Hepatocyte apoptosis is ubiquitous in liver diseases. Although apoptosis is primarily a non-inflammatory process responsible for removing excess or damaged cells, un-controlled apoptosis can deteriorate organ function. E.g. if apoptotic bodies are not eliminated, their membranes become permeable, leading to the release of cellular fragments into the extracellular space and triggering an inflammatory response, a process called secondary necrosis. In massive liver injury, the ability of phagocytes to identify and clear apoptotic bodies is likely disturbed and an inflammatory response is observed despite an initial apoptotic stimulus in the liver. Therefore, understanding the cellular processes and molecular signaling pathways regulating apoptosis and/or necrosis in hepatocytes is essential to the development of new therapeutic strategies for (chronic) liver diseases. In particular, the intracellular organelles and membrane receptors that are involved in hepatocyte cell death and their interactions are of substantial interest, as a single toxic stimulus often activates several intracellular apoptotic pathways simultaneously. In this review, we discuss recent advances in organelle-mediated and membrane receptor-mediated cell death and potential targets for therapy in hepatocytes.

Keywords: Hepatocyte; Apoptosis; Receptors

Introduction

Liver diseases belong to the top 10 of leading diseases for humans. In clinical practice, liver injury is divided into acute and chronic diseases, based on the duration or persistence of liver injury. After recovery from acute liver injuries, normal liver function and architecture are restored. In contrast, liver functions in most chronic liver injuries are abnormal and persistent changes in liver architecture occur. Hepatocytes comprise 80% of all liver cells and hepatocyte injury is often central in the pathogenesis of liver diseases [1]. Prolonged hepatocyte injury results in an excessive wound healing response, leading to hepatic inflammation and fibrogenesis. Liver fibrosis is the hallmark of chronic liver diseases that may progress to end-stage liver disease and hepatocellular carcinoma. Toxic bile salts, cytokines, reactive oxygen species, free fatty acids and drugs induce hepatocyte injury.

Hepatocyte injury can result in hepatocyte cell death via apoptosis and/or necrosis [2,3]. Necrosis is a passive process that associates with metabolic disruption, energy depletion (loss of ATP), mitochondrial swelling and rupture of the plasma membrane. Subsequently, the release of cellular content into the extracellular environment and systemic circulation triggers an inflammatory response in the liver. Apoptosis is an ATP-dependent process also known as programmed cell death. Apoptosis is characterized by DNA condensation, nuclear fragmentation, plasma membrane blebbing, cell shrinkage and the formation of apoptotic bodies. In the liver, surrounding phagocytosing cells clear apoptotic bodies and limit the inflammatory response [2-5].

Although apoptosis is primarily a non-inflammatory process responsible for removing excess or damaged cells, un-controlled apoptosis (as in pathologic conditions) can deteriorate organ function [6]. Hepatocyte apoptosis is ubiquitous in liver diseases [1,7-11]. In the liver, apoptosis contributes to inflammation by promoting the activation of Kupffer cells and myofibroblasts. Following the uptake of apoptotic bodies, Kupffer cells express death ligands such as TNFα, TRAIL and FasL [12]. All these death ligands may induce apoptosis in hepatocytes via death-receptor induced signaling cascades and thus aggravate liver injury [3,13,14]. In addition, activated myofibroblast

(derived from portal fibroblasts and HSCs [15,16]) are able to engulf apoptotic bodies and subsequently produce profibrogenic factors such as TGFβ and type I collagen [17,18]. These data link hepatocyte apoptosis to liver inflammation and liver fibrosis in chronic liver diseases and suggest that the most direct therapeutic strategy for repressing liver damage is to eliminate the cause of hepatocyte injury. For example, many chronic hepatitis B patients with end-stage liver disease had significant recovery and reversal of liver fibrosis with antiviral therapy and no longer required urgent transplantation (reviewed by [19]). Therefore, removing the cause for hepatocyte injury has become a potential therapeutic strategy for advanced liver diseases. However, effective treatments do not exist for many liver diseases such as primary sclerosing cholangitis, NASH, ASH and patients with chronic HCV or HBV unresponsive to antiviral therapies. For such patients anti-apoptotic strategies, reducing hepatocyte cell death-mediated inflammation and fibrogenesis, are beneficial [20-22]. Thus, understanding the cellular processes and molecular signaling pathways mediating hepatocyte apoptosis is essential to the development of new therapeutic strategies. In particular, the intracellular organelles and membrane receptors that are involved in hepatocyte cell death and their interactions are of substantial interest, as a single toxic stimulus often activates several intracellular apoptotic pathways simultaneously. In this review, we discuss recent advances in organelle- and membrane receptor-mediated cell death and potential targets for therapy in hepatocytes.

Organelle-mediated cell death

Mitochondria: Mitochondrial dysfunction is a common observation in several acute and chronic liver diseases such as ASH, NAFLD, drug-induced hepatotoxicity, viral hepatitis, biliary cirrhosis, hepatocellular carcinoma, ischemia/reperfusion injury and transplant rejection [23]. Mitochondria play an essential role in regulating the intrinsic pathway of hepatocyte apoptosis as well as hepatocyte necrosis [3,24-28]. Mitochondrial Permeability Transition (MPT) is a key mechanism underlying both apoptosis and necrosis (Figure 1). MPT is characterized by an increase in the permeability of the inner mitochondrial membrane, resulting in the loss of membrane potential, mitochondrial swelling and the rupture of the outer mitochondrial membrane [26]. Opening of the Permeability Transition Pore (mPTP) in the mitochondrial inner membrane is suggested to initiate the MPT. In addition, outer membrane channels such as Mitochondrial Apoptosis-Induced Channel (MAC) and the Voltage Dependent Anion Channel (VDAC), directly or indirectly, are involved in mitochondrial permeabilization during apoptosis and/or necrosis (Figure 1) [29]. Mitochondrial Outer Membrane Permeabilization (MOMP) during intrinsic apoptosis leads to the release of apoptotic factors such as cytochrome c, Second Mitochondrial Activator of Caspase/Direct IAP Binding protein with Low pI (SMAC/DIABLO), High-Temperature Requirement protein A2 (HtrA2/Omi), Apoptosis-Inducing Factor (AIF) and endonuclease G [30,31]. Subsequent activation of effector caspases leads to the proteolysis and typical morphological changes of apoptosis [32]. Although the molecular composition of the mPTP is not completely known, three components are suggested to be related to the mPTP (directly or indirectly): the outer membrane channel VDAC [33-35], the Adenine Nucleotide Translocator (ANT) [36] and cyclophilin-D [37]. However, studies with knockout animals have raised doubt about the involvement of ANT and VDAC in the mPTP [38,39]. In addition, recent studies with cyclophilin-D deficient cells strongly suggest that mPTP opening is a consequence, rather than the cause for apoptosis, and validate the original proposal that the mPTP is key in necrotic cell death (Figure 1) [37,40,41]. Furthermore, the observation that cytochrome c release can occur without the loss of outer membrane integrity indicates that a more selective mechanism of permeabilization such as the formation of a pore in the outer membrane instead of membrane rupture is operating in apoptosis [42-45]. The MAC forms early in apoptosis in the mitochondrial outer membrane and provides a direct pathway for the release of cytochrome c from the inter-membrane space into the cytosol. Bcl-2 family proteins tightly regulate the MAC and pro-apoptotic members of Bcl-2 family such as Bak and Bax are components of this channel (Figure 1) [31,42,43,45-47]. However, the complete molecular structure of the MAC is not known and it is suggested that the MAC may contain additional components [31,48]. Both the mPTP and the MAC are potential therapeutic targets to induce cell death in malignancies and prevent cell death in degenerative and ischemia-associated pathologies. Indeed, NIM811 (a non-immunosuppressor cyclosporine-analogue) is reported to decrease liver injury and induce liver regeneration by inhibiting MPT after liver transplantation or massive hepatectomy [49,50]. NIM811 is also reported to attenuate cholestatic necrosis and apoptosis in BDL rats via inhibition of MPT [51]. Modulation of MPT in a multidrug-resistant hepatocellular carcinoma cell line with the selective MPT opener, Atractyloside Glycoside (ATR), was shown to increase apoptosis in these cells whereas the selective inhibitor of MPT, Cyclosporine A, had the opposite effect [52]. These data suggest that targeting MPT is a potential therapeutic strategy for different liver diseases.

Endoplasmic reticulum: Endoplasmic Reticulum (ER) stress is suggested to be an important mechanism in the pathogenesis of chronic liver diseases including NAFLD, ASH, viral hepatitis, drug-induced liver injury, ischemia/reperfusion injury and cholestatic liver disease [53]. The ER is responsible for synthesis, folding, trafficking and maturation of proteins. Under pathologic conditions, the homeostatic equilibrium between the influx of the unfolded proteins and the folding capacity of the ER is disturbed. This leads to the activation of signal transduction pathways between the ER and other intracellular organelles to mediate cellular adaptation to new demands (Figure 2). These series of compensatory responses, termed Unfolded Protein Response (UPR), are conserved in the evolution to promote cellular survival [54]. In addition, glucose deprivation and the depletion of calcium stores can induce ER-stress [55-57]. Three membrane sensors in the ER mediate the ER-stress signal transduction: inositol-requiring enzyme-1α (IRE1α), Activating Transcription Factor (ATF) 6 and Protein kinase RNA-like Endoplasmic Reticulum Kinase (PERK) (Figure 2). These transmembrane sensors are kept inactivate as long as they are bound to the intraluminal chaperone Glucose-Regulated Protein 78 (GRP78) (Figure 2) [58,59]. IRE1α is an endoribonuclease that promotes the splicing of X-box Binding Protein 1 (XBP1) mRNA, resulting in transcription of UPR elements and ER-stress response genes that control ER-Associated Protein Degradation (ERAD) and chaperones [60-62]. PERK induces phosphorylation of eukaryotic translation initiation factor-2α subunit (eIF2α), thereby globally inhibiting protein synthesis [63]. PERK also regulates the transcription of ribosomal RNA via phosphorylation of eIF2 and increases the translation of ATF4. In turn, ATF4 binds to the cAMP-Response Element (CRE) resulting in the synthesis of C/EBP (CCAAT/enhancer binding protein) Homologous Protein (CHOP) [58,64,65]. Active ATF6 translocates to the nucleus and together with sXBP1 and ATF4, activate ER-stress response elements, UPR elements and CRE [66-68]. Upon ER-stress, GRP78 is displaced from the stress sensor, leading to the activation of these three ER-stress mediated signaling pathways (i.e. IRE1α, ATF6 and PERK) (Figure 2). Prolonged or unchecked ER-stress leads to steatosis, apoptosis and inflammation in the liver
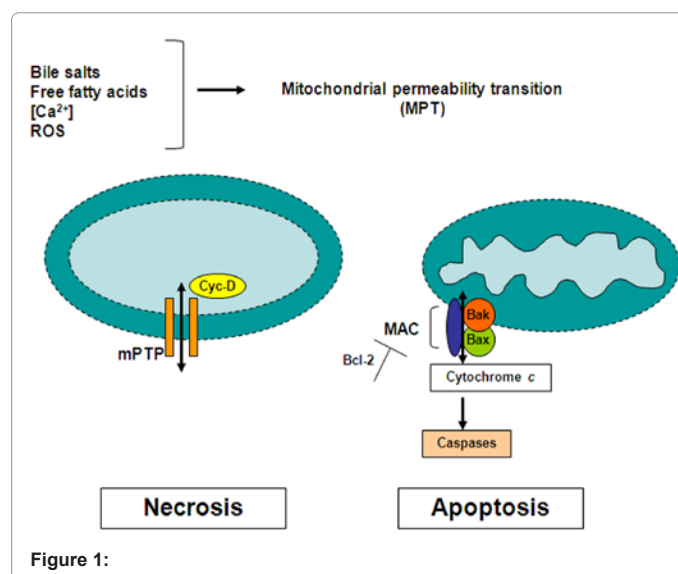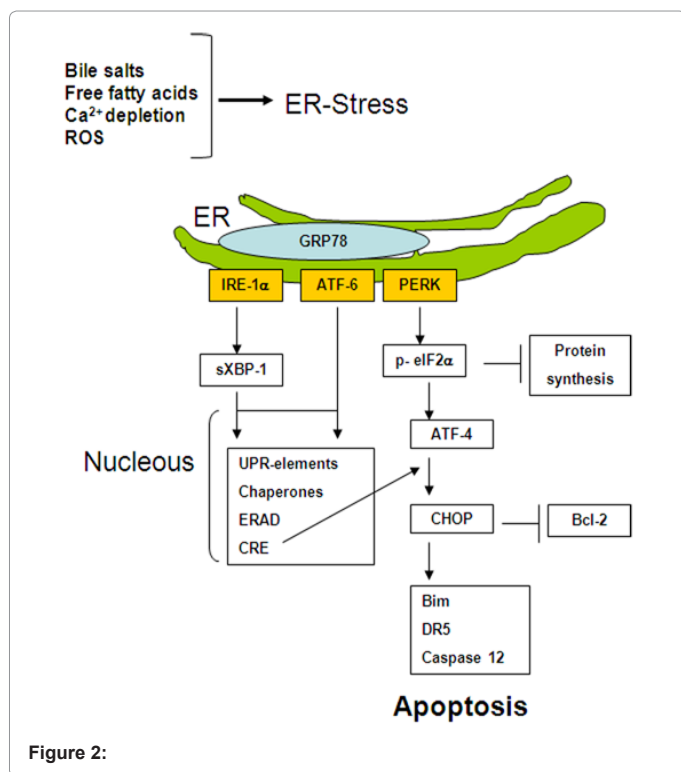

Figure 1:

Figure 2:

[53]. An important feature of the ER-stress response is increased CHOP expression leading to the activation of proapoptotic pathways [69]. Overexpression or microinjection of CHOP protein has been reported to promote cell cycle arrest and/or apoptosis [70,71]. CHOP can induce the expression of proapoptotic BH3-only protein Bim and the cell surface death receptor TRAIL receptor 2 (also known as death-receptor 5, DR5) and inhibit Bcl-2 transcription [72-74]. CHOP-knockout mice are protected against alcohol-induced hepatocyte apoptosis after alcohol feeding as well as against bile salt-induced hepatocyte apoptosis after bile duct ligation [75,76]. Acetaminophen (APAP) intoxication has been observed to induce CHOP expression and to cause an intraluminal redox imbalance of the ER, resulting in hepatocyte apoptosis [77]. Importantly, the role of ER stress in APAP-induced necrosis is unknown. However, a role for ER stress in the early activation of JNK and in Ca^{2+}-mediated mitochondrial permeability transition, both key factors in APAP-induced necrosis, could be considered [78,79].

Several therapeutic interventions could modulate ER-stress for example chemical chaperones that ameliorate protein folding and antioxidants that counteract oxidative stress. The chemical chaperone 4-Phenylbutyrate (4PBA) is able to reduce the ER-stress response and decrease ER-associated caspase 12 activation in livers of mice undergoing ischemia-reperfusion injury [80]. However, since caspase 12 is only present in mice, ER-stress may activate other apoptotic pathways in human hepatocytes, such as Ca^{2+}-mediated mitochondrial permeability transition. CHOP antagonism is also an obvious target for therapy as CHOP is involved in oxidative stress and apoptosis in hepatocytes. Improved protein folding would be beneficial in disorders of misfolded proteins such as alpha-1-antitrypsin deficiency. Thus, agents that ameliorate ER-stress by promoting adaptive UPR signaling or inhibiting ER-stress response-induced apoptosis offer a therapeutic

opportunity. Since the ER-stress response has extensive cross-talk with other stress responses, therapeutics aimed at blunting the ER stress response may interrupt this inter-organelle cross-talk that operates in many liver diseases [53].

Lysosomes: Lysosomes are involved in necrotic, apoptotic and autophagic cell death. The key factor in determining the type of cell death is the magnitude of Lysosomal Membrane Permeabilization (LMP) and the amount of proteolytic enzymes released into the cytosol [81]. Massive breakdown of lysosomes results in unregulated necrosis, whereas selective permeabilization of lysosomes triggers apoptosis. Several mechanisms for the controlled permeabilization of lysosmes have been proposed (Figure 3). One theory includes the accumulation of lysosomotropic detergents such as sphingosine in the lysosomes, facilitating the release of lysosomal enzymes into the cytoplasm [82]. Another theory involves ROS-mediated lysosomal destabilization. In this theory, LMP is suggested to precede mitochondrial dysfunction, thereby creating a feedback loop between mitochondrial-derived ROS and LMP to control cell death (Figure 3). In addition, intralysosomal accumulation of free iron indirectly mediates lysosmal membrane damage via generation of ROS [83,84]. Translocation of proapoptotic members of the Bcl-2 family such as Bax and Bim to the lysosomes, leading to pore formation and membrane permeabilization (similar to their role in mitochondrial permeabilization) is also proposed as a mechanism for lysosomal leakage (Figure 3) [85-88]. LMP associated with cathepsin translocation may directly activate calpains and caspases, triggering classic MOMP- and caspase-dependent apoptosis and/or caspase-independent cell death [89]. Increased lysosomal enzyme activity (e.g., acidic phosphatase) is observed in patients with chronic liver diseases including chronic viral hepatitis, cirrhosis and hepatocellular carcinoma [90]. In addition, LMP and cathepsins have been implicated in cell death in several models of liver injury. For instance, it has been shown that intracellular levels of sphingosine in the liver increases after TNFα treatment, leading to LMP and apoptosis. Interestingly, TNFα or sphingosine could not induce LMP in hepatocytes from cathepsin-B knockout livers, suggesting that cathepsins may also be inducers of LMP. Cathepsins may directly induce LMP acting from the inside and/or outside of the lysosomes or they may participate in an amplification loop in which LMP induces cathepsin activation, and cathepsin then triggers further LMP (Figure 3) [88,91]. Free fatty acids and bile salts can also induce LMP-dependent cell death [92-95]. Glycochenodexycholic acid (a cholestatic bile salt) induces LMP, cathepsin-B translocation, caspase activation and cell death in hepatocytes in animal models of cholestasis [92,96].
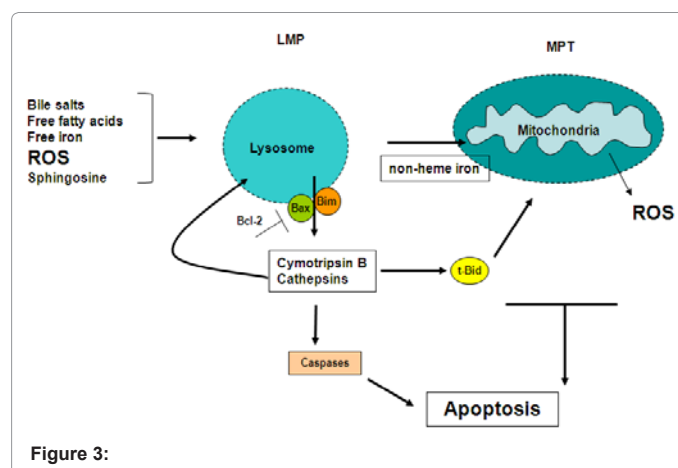

Figure 3:

Excessive accumulation of saturated free fatty acids in the liver has been reported to directly induce mitochondrial dysfunction and oxidative stress via LMP and activation of cathepsin B [95]. Translocation of non-heme iron from lysosomes to the mitochondria is reported to play an important role in oxidative-stress induced hepatocellular damage, identifying this pathway as a potential therapeutic target to combat oxidative stress-mediated hepatotoxicity (Figure 3) [97]. Cymotrypsin B, a lysosomal enzyme originally described in pancreas, was also found in rat liver lysosomes. Interestingly, Cymotrypsin B was reported to cleave the non-apoptotic Bid into pro-apoptotic Truncated Bid (tBid) at neutral pH.tBid then translocates to mitochondria, leading to MOMP and cytochrome c release (Figure 3). Knockdown of Cymotrypsin B or pretreatment with the Cymotrypsin B inhibitor, N-p-tosyl-L-phenylalanine chloromethyl ketone, reduced TNFα-induced apoptosis in hepatocytes [98]. As noted before for ER-stress, it is important to appreciate that disturbance in redox status, steatosis, oxidative stress, inflammation and mitochondrial injury all affect LMP. LMP can activate interorganellar signaling pathways, death-receptor mediated signaling and lysosomal proteases such as cathepsin B. Therefore, therapeutics aimed at reducing LMP may interrupt all these signaling pathways, thereby reducing liver inflammation and hepatocyte damage.

Receptor-mediated cell death

Membrane death receptors: Death receptors belong to the TNF/Nerve Growth Factor superfamily and are involved in death ligand-mediated cell death. TNFα, Fas ligand (FasL) and Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) are death ligands that signal via binding to their membrane-bound receptors including: Fas, Tumor Necrosis Factor Receptor (TNFR1 and TNFR2), and TRAIL receptors (R1/DR4 and R2/DR5). Receptor-ligand binding triggers receptor trimerization at the cell surface, providing a platform at the cytoplasmic side of the plasma membrane (termed Death Domains) which recruits adaptor proteins such as Fas-Associated Death Domain (FADD). However, the exact composition of a functional TNFR as the initial trigger of signal transduction is a matter of dispute. Indeed, recent data suggest that a receptor dimer is a minimum functional unit and upon ligand induced-clustering of receptors large polymeric complexes assemble to induce signal transduction pathways [99,100]. The interaction between the Death Domains (DD) of death receptors and adaptor proteins leads to the activation of caspase-8 and subsequent cleavage of Bid to tBid. Translocation of tBid to mitochondria results in mitochondrial permeabilization and activation of the apoptotic cascades. In addition, TNFα-induced Bid-dependent and TRAIL-induced Bax-dependent LMP has been described. LMP then leads to cell death [88,101]. Although death receptors do not induce ER stress, ER stress-mediated regulation of TRAIL receptor (DR5) expression has been reported [73,102]. Therefore, interaction between death receptor signaling and intracellular organelles plays an important role in cell death (Figure 4). Hepatocytes in particular, characterized as type II cells, require the mitochondrial induced-apoptotic cascades coupling to the activation of death receptor induced-apoptotic pathways in order to conduct an effective apoptotic signal.

Several liver cells including hepatocytes express Fas (CD95/ Apo-1) [103-105]. Fas is activated upon binding of membrane-bound FasL (mFasL) or soluble FasL (sFasL). mFasL is expressed on several immune cells including cytotoxic T lymphocytes and natural killer (NK) cells [106]. mFasL then activates Fas-mediated cell death, leading to the removal of unwanted hepatocytes such as virus-infected hepatocytes

and cancer cells by immune cells [107]. While mFasL is a proapoptotic trigger, sFasL is an antagonist for mFasL-induced apoptosis. It was shown that T-cells of mice lacking sFasL are able to kill target cells via Fas-induced apoptosis whereas T-cells of mice lacking mFasL were incompetent to induce Fas-induced apoptosis [108]. Indeed conversion of mFasL to sFasL by metalloproteinase (a so-called shedding process) is a mechanism to prevent the killing of adjacent healthy cells by cytotoxic immune cells [109,110]. Nevertheless, excessive Fas-induced cell death leads to liver failure. Indeed, injection of Fas agonistic antibody to mice induces fulminant hepatic failure. In addition, it was shown that anti-apoptotic and pro-apoptotic Bcl-2 members regulate this toxicity [111-113]. Elevation of soluble FasL occurs in patients with acute liver failure (such as drug-induced liver injury and acetaminophen-induced liver injury), which may be a defensive mechanism of the liver to reduce the Fas-induced liver toxicity. In addition,FasL and/or Fas receptor expression is increased in many chronic liver diseases including chronic viral hepatitis and alcoholic liver diseases [114-117]. In summary, Fas/FasL induced apoptotic cell death plays a crucial role in liver pathogenesis.

TNFR1 and TNFR2 are both expressed on hepatocytes, but only TNFR1 expresses a DD [118]. Importantly, TNFR1 activation in hepatocytes can trigger both apoptotic and survival signaling. TNFα-exposure leads to the rapid formation of a DISC (Death Inducing Signaling Complex) composed of TNFα, the TNFR-Associated Death Domain Adaptor Molecule (TRADD), the Fas-Associated Death Domain Adaptor Molecule (FADD), caspase-8, TNFR-Associated Factor -2 (TRAF2) and Receptor-Interacting Protein (RIP). Interestingly, it was shown that TNFR1 and some DISC components also appear inside mitochondria within 30 minutes after TNFα-exposure, suggesting that TNFα-mediated signaling includes the translocation of TNFR1 (and associated proteins) to mitochondria [119]. In contrast, TRADD suppresses apoptosis by recruiting RIP, TRAF2 and TRAF5. Immediate binding of RIP and TRAF2 to TRADD lead to the activation of Nuclear Factor-κB (NF-κB) and transcriptional activation of pro-survival genes including Bcl-xL, A1, XIAP and cFLIP [120,121]. TNFα/TNFR2 signaling is involved in the activation of hepatocyte DNA synthesis and proliferation as well as in the regulation of FasL-dependent clearance of virus infected hepatocytes by Cytotoxic T Lymphocytes (CTL) [122,123]. Interestingly, mice lacking both TNFR1 and TNFR2 are resistant to anti-Fas induced-fulminant hepatic failure [124]. These data suggest that TNFα/TNFRs interactions are critical for the proper functioning of CTL activity in the liver and clearance of infected and/or damaged hepatocytes.

TRAIL is emerging as a key mediator of hepatic injury during inflammatory disorders of the liver [125]. Early studies demonstrated that different cells have different sensitivity to TRAIL-induced cell death. Whereas cancer cells are sensitive to TRAIL-induced toxicity, normal (non-transformed) cells were resistant to its apoptosis-inducing effects. Hence, TRAIL-induced selective cancer cell death without damage to the adjacent normal tissue was suggested to be an efficient anti-cancer therapy despite that many cancer cells are now considered as resistant to TRAIL induced-apoptosis [126,127]. Indeed, TRAIL, DR4 and DR5 (TRAIL receptors) messenger RNA are expressed at low levels in normal human liver and the expression of the receptors at the protein level is difficult to detect. However, emerging data indicate that DR4 and DR5 expression is up-regulated in several liver diseases such as steatosis and HCV- and HBV-infection [128-131]. HIV and/or the ligation of HIV glycoprotein gp120 to CXCR4

on hepatocytes also selectively increases DR5 expression and suggest that HIV infection renders hepatocytes more susceptible to apoptotic cell death in liver diseases associated with enhanced TRAIL expression such as HBV, HCV or steatohepatitis [132]. Increased DR5 expression in experimental models of NASH is associated with the activation of p53 and ER-stress induced CHOP expression by free fatty acids such as palmitate [133,134]. In addition, bile acids can increase DR5 expression and inhibit cFLIP (inhibitor of DR5-induced signaling) function, thereby sensitize hepatocytes to TRAIL mediated cell death in cholestatic livers [135,136]. Thus, the safety of TRAIL administration to humans with underlying liver disease is questionable. The development of selective DR4 agonistic antibodies to treat cancer in patients with underlying liver diseases could be an attractive strategy, considering the possibility of DR5-dependent TRAIL toxicity in liver diseases.

In summary, excessive generation of inflammatory mediators and activation of death receptor-mediated apoptotic pathways can exacerbate underlying liver diseases. Therefore, therapies targeting death receptor-mediated hepatotoxicity in combination with efforts to remove the cause of the disease (e.g., virus, toxic bile acids, free fatty acids and drugs) may be a beneficial strategy in the treatment of liver diseases.

Epidermal growth factor receptor: The epidermal growth factor receptors (EGFR; ErbB-1; HER1 in humans) are the cell-surface receptors for members of the Epidermal Growth Factor Family (EGF-family) of extracellular protein ligands. The EGFR is a member of the ErbB family of receptors, a subfamily of four closely related receptor tyrosine kinases: EGFR (ErbB-1), HER2/c-neu (ErbB-2), Her 3 (ErbB-3) and Her 4 (ErbB-4) [137]. Growth factor receptors such as the EGFR are involved in both cell proliferation and cell death in the liver [138]. EGF functions as an endocrine factor. It is produced by a variety of cells and EGF/EGFR interaction is essential for liver regeneration [139]. Ligand-dependent EGFR activation also plays a role in hepatic tumorigenesis and EGFR-targeting agents such as monoclonal antibodies (e.g., cetuximab and panitumumab) and tyrosine kinase inhibitors (e.g., gefitinib and erlotinib) are suggested in the treatment of liver carcinomas [140,141]. In addition to ligand-dependent EGFR activation, ligand-independent EGFR activation in the liver has been described. For instance, hydrophobic bile acids induce Reactive Oxygen Species (ROS)-dependent EGFR tyrosine phosphorylation in hepatocytes, leading to the activation of downstream Mitogen-Activated Protein Kinases (MAPK) [14]. Hydrophobic bile acids also induce Extracellular Kinase Regulated (ERK)-dependent Hepatic Stellate Cell (HSC) proliferation via ROS-dependent EGFR activation [142]. As described above, ligand-independent EGFR activation plays an important role in liver pathogenesis. In hepatocytes, ligand-independent EGFR activation also contributes to apoptosis [138]. Several mechanisms for ligand-independent EGFR activation have been described such as EGFR transactivation by Fas ligand (FasL)[143,144], hydrophobic bile salts (deoxycholate, glychocenodeoxycholate, etc) or hyperosmolarity [14,143,144] (Figure 4). Ligand-independent activation of EGFR, a process that requires ROS-dependent Yes activation (a non-receptor tyrosine kinase protein of Src kinase family) followed by Yes-mediated EGFR transactivation [143-147], follows JNK-dependent EGFR/Fas association. EGFR/Fas association results in EGFR-mediated tyrosine phosphorylation of Fas (death receptor), leading to Fas oligomerization, membrane translocation, DISC formation and execution of apoptosis [143,144,148].

These data suggest that agents that inhibit ligand-independent activation of EGFR such as tyrosine kinase inhibitors may have substantial anti-apoptotic effects in hepatocytes. Interestingly, recent evidence suggests that the EGFR can also function as a host cofactor for HCV entry and tyrosine kinase inhibitors show substantial antiviral activity [149]. Chronic viral hepatitis is associated with activation of inflammatory mediators and accumulation of immune cells in the liver, which can induce hepatocyte apoptosis/necrosis in non-infected hepatocytes via activation of death-receptor signaling pathways. Thus inhibition of receptor tyrosine kinase (such as EGFR) may constitute an attractive novel approach in the treatment of chronic liver diseases, in particular chronic viral hepatitis due to their antiviral activity and anti-apoptotic effects in healthy hepatocytes.

G-protein coupled receptors: GPCRs are the largest family of membrane proteins. More than 300 GPCRs have been reported in humans and rodents [150]. GPCRs transduce extracellular signals to intracellular effector pathways. Upon activation by agonists, GPCRs activate heterotrimeric G-proteins ($G_\alpha\beta\gamma$). These subunits subsequently activate second messengers (e.g. cAMP, Ca^{2+} and protein kinases), relaying the GPCR induced-signal to the intracellular targets. Heterotrimeric G-proteins are divided into 4 families (i.e., Gαs, Gαi, Gαq/11 and Gα12/13) based on the G_α subunit sequence and signaling activity [151]. Many GPCRs such as lysophosphatidic acid (LPA), sphingosine-1 phosphate (S1P) and orexin (OXR) receptors are involved in the regulation of apoptosis in cancer cells [152-154]. Signaling components, acting downstream of the GPCRs or G-proteins (e.g., arrestin and adaptor protein 2) may mediate anti-apoptotic events following stimulation of GPCRs [155,156]. Activation of GPCRs regulates apoptosis in cancer cells via interaction with different intracellular regulators of apoptosis such as MAPKs, NF-κB and p53-associated pathways [157]. E.g., LPA-dependent signaling decreases the nuclear localization and cellular abundance of p53, leading to resistance of lung carcinoma cells to apoptosis [158]. Activation of the receptors for LPA, ET1 (endothelin) and angiotensin II can activate NF-κB-regulated pathways. NF-κB then mediates either anti-apoptotic or pro-apoptotic responses, depending on the stimulus and the cell type [120,159-162]. In the liver, activation of GPCRs also mediates both apoptotic and anti-apoptotic responses. E.g., S1P receptors mediate both apoptotic and anti-apoptotic pathways in human hepatic myofibroblasts [163]. Free fatty acids such as palmitic
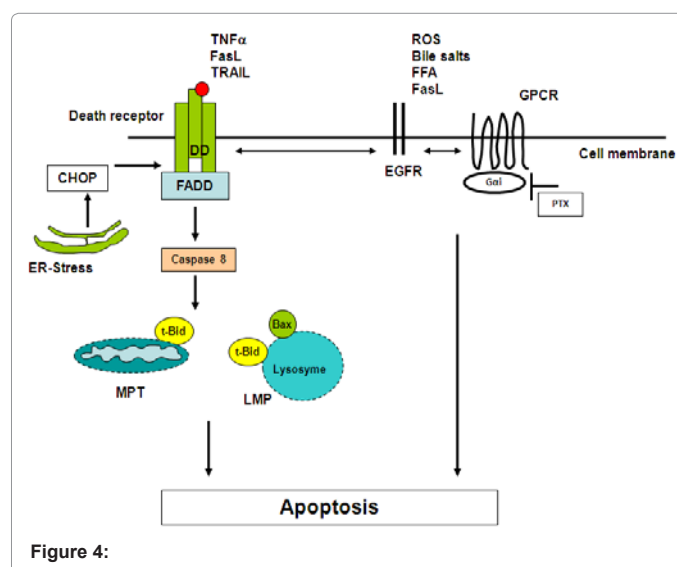

Figure 4:

acid mediate apoptosis in hepatocytes via phospholipase A2 (PLA_2)/lysophosphatidylcholine (LPC)/LPA-dependent signaling [164]. However, the exact signaling pathways downstream of GPCRs that regulate apoptosis in hepatocytes are yet unknown. Nevertheless, some GPCR-based drugs show potent anti-tumor efficacy e.g. the endothelin A receptor antagonists -ZD4054 and atrasentan [157,165-167]. GPCR-based drugs may also show therapeutic benefits in the regulation of apoptosis in chronic liver diseases and liver tumors. We have shown that Pertussis Toxin (PTX), an inhibitor of G_α-protein, protects hepatocytes but not hepatocellular carcinoma cells against bile salt-induced and cytokine-induced apoptosis, suggesting a cross-talk between GPCR and EGFR in hepatocyte apoptosis (Figure 4) [168]. Activating death receptor-mediated apoptosis in cancer cells by death ligands is suggested as an effective therapeutic strategy in the treatment of hepatocellular carcinoma [169] but this strategy may lead to the induction of apoptosis in adjacent normal hepatocytes. Therefore, an adjuvant anti-apoptotic therapy that will inhibit cell death in normal hepatocytes but has no effect on cancer cells may prevent excessive liver damage. Our data suggest that GPCR/$G_{\alpha i}$-based therapeutic strategies may serve as the anti-apoptotic adjuvant therapy, protecting normal tissue against inflammation, bile salt and/or drug-induced cell death.

Conclusions

Understanding the cellular mechanisms that control death in hepatocytes is of clinical and scientific importance in the development of novel therapies. Hepatocyte cell death appears to be at the crossroads of several cellular mechanisms rather than the end-point of a linear cascade of events. One particular toxic stimulus may simultaneously promote the activation of several types of membrane-bound receptors as well as organelle-mediated signaling pathways, leading to apoptosis. In addition, several toxic stimuli often play a role in the pathogenesis of chronic liver diseases (e.g., free fatty acids and inflammatory mediators in NASH). Thus, anti-apoptotic therapeutics aimed at one particular pathway may be more effective when used in combination with other interventions.

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