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Apoptosis in liver diseases – detection and therapeutic applications

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Summary

The liver is continuously exposed to a large antigenic load that includes pathogens, toxins, tumor cells and dietary antigens. Amongst the hepatitis viruses, only hepatitis B virus (HBV) and hepatitis C virus (HCV) cause chronic hepatitis, which can progress to cirrhosis and hepatocellular carcinoma. Of the different antiviral defense systems employed by the tissue, apoptosis significantly contributes to the prevention of viral replication, dissemination, and persistence. Loss of tolerance to the liver autoantigens may result in autoimmune hepatitis (AIH). This review outlines the recent findings that highlight the role and mechanisms of apoptotic processes in the course of liver diseases. Among factors that contribute to liver pathology, we discuss the role of tumor necrosis factor (TNF)-α, HBx, ds-PKR, TRAIL, FasL, and IL-1α. Since TNF and FasL-induced hepatocyte apoptosis is implicated in a wide range of liver diseases, including viral hepatitis, alcoholic hepatitis, ischemia/reperfusion liver injury, and fulminant hepatic failure, these items will be discussed in greater detail in this review. We also highlight some recent discoveries that pave the way for the development of new therapeutic strategies by protecting hepatocytes (for example by employing Bcl-2, Bcl-X_L or A1/Bfl-1, IAPs, or synthetic caspase inhibitors), or by the induction of apoptosis in stellate cells. The assessment of the severity of liver disease, as well as monitoring of patients with chronic liver disease, remains a major challenge in clinical hepatology practice. Therefore, a separate chapter is devoted to a novel cytochrome c – based method useful for the diagnosis and monitoring of fulminant hepatitis.

key words: **apoptosis • cirrhosis • cytochrome c • death receptors • hepatitis • mitochondrial death pathway**

Abbreviations: **CIDE** – cell death-inducing DFF45-like effector protein; **HBX** – Hepatitis B X protein; **HCV-NS2** – hepatitis C virus non-structural protein 2; **IAP** – inhibitor of apoptosis protein; **MMP** – mitochondrial membrane permeabilization; **MPT** – mitochondrial permeability transition; **tBid** – truncated BH3-interacting domain death agonist; **TNF** – tumor necrosis factor-α; **TNFR** – TNF receptor; **VDAC** – voltage-dependent anion channel

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BACKGROUND

The liver is exposed to many potentially harmful agents that, under normal conditions, do not damage the liver cells due to the protective mechanisms and the large repair capacity of hepatocytes. However, acute or chronic exposure to insults such as cytokines, reactive oxygen species and bile acids results in dissipated liver function. Both cell proliferation and apoptosis are required for proper development of the biliary tree and parenchyma of the liver [1].

During acute and chronic liver diseases, hepatocytes are exposed to increased levels of cytokines like tumour necrosis factor-α (TNF)-α, interleukin-1α (IL-1α) and interferon-γ (IFN-γ), oxidative stress and bile acids [2]. Although hepatocytes have an abundant capacity to defend themselves against these agents, excessive exposure will lead to cell death. Hepatic cell death occurs in both acute and chronic liver diseases. Therefore deep insight into the cellular mechanisms leading to cell death is of utmost importance in understanding the pathophysiology of liver diseases.

Based on the morphology and biochemical changes, cell death can be divided into at least into two different processes: necrosis and apoptosis, although intermediate forms of cell death also occur. However, characteristic features of both necrotic and apoptotic cell death can occur in the same tissue and even in the same cell concomitantly [3]. Necrosis results from metabolic disruption in parallel with energy depletion (loss of ATP), mitochondrial and cellular swelling and activation of enzymes that degrade cellular structures. This leads to cell lysis, followed by the discharging of cell constituents into the surrounding microenvironment. Therefore necrosis is accompanied by inflammation because of immune system effector cell recruitment. In contrast, apoptotic cell death is ATP-dependent, and in comparison with necrosis, does not stimulate an immune response [4]. Apoptosis is characterized by DNA condensation, nuclear fragmentation, plasma membrane blebbing and cell shrinkage. Eventually, the apoptotic cell breaks into small membrane-surrounded fragments (apoptotic bodies) that are cleared by scavenging macrophages and neighbor cells [5,6]. All these events are strictly controlled and well organized. Both apoptosis and necrosis almost always occur together in intact organisms; however, the relative contribution of the different modes of cell death may vary [7]. Apoptosis can be induced by a vide variety of internal and external insults, including deregulation of cellular metabolic pathways, cytokines, viruses, and anticancer drugs [8–14]. Apoptotic cell death has been reported in liver diseases, in particular acute liver injury [15], and also in chronic liver diseases, although the actual significance of apoptotic cell death in chronic liver diseases remains to be determined [16].

Apoptosis contributes to the elimination of damaged, mutated, aged, or virally infected cells [17–19]. Apoptosis may be initiated by the extrinsic pathway, in which death receptors expressed on the cell surface trigger the receptor-proximal activation of adapter molecules and caspases and later the dissipation of mitochondrial membrane permeabilization [6,20]. When apoptosis is triggered by the intrinsic pathway, death signals act directly on mitochondria leading to mitochondrial membrane permeabilization before caspases are activated [21]. The permeabilization of mitochondrial membranes leads to the release of pro-apoptotic factors, some of which can activate caspases, a family of proteins that serve as a cellular demolition system [22–24], whereas others can activate caspase-independent death pathways [25–27]. Mitochondrial membrane permeabilization is tightly regulated by proteins from the Bcl-2 family, which inhibit or promote mitochondrial membrane permeabilization, depending on whether they belong to the pro- or anti-apoptotic branch of the family, respectively [28]. Mitochondrial membrane permeabilization thus frequently marks the point-of-no return of the apoptotic process, the point beyond which cells succumb to death [29]. The intrinsic or mitochondrial pathway is activated by a variety of extra- and intracellular stressors, including hypoxic conditions and treatment with cytotoxic drugs [19,30].

APOPTOSIS IN LIVER DISEASE

Apoptotic cell death of hepatocytes emerges as a fundamental component of virtually all acute and chronic liver diseases. The liver tissue repair, inflammation, regeneration, and fibrosis may all be triggered by apoptosis [2,31,32]. Of these processes, hepatic fibrosis has the potential to be the most deleterious, as progressive fibrosis can culminate into cirrhosis with portal hypertension and chronic liver failure. An increasing body of evidence from both experimental and clinical studies suggests that hepatocyte apoptosis may contribute to liver fibrogenesis [33]. For instance, in animal models of cholestasis, attenuation of hepatocyte apoptosis also reduces fibrogenesis [34]. Engulfment of apoptotic bodies by hepatic stellate cells stimulates the fibrogenic activity of these cells and may be one mechanism by which hepatocyte apoptosis promotes fibrosis [34]. Hepatocyte apoptosis can be induced through the death receptor-dependent pathway (extrinsic pathway) or the mitochondrial-dependent pathway (intrinsic pathway). The death receptor-dependent pathway is initiated in the liver by death ligands like TNF, Fas ligand (FasL, CD95L), and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), following their binding to their relevant death receptors. Among them, TNF and Fas ligand are being considered as the major players and thus they have been extensively studied. Death receptors are expressed on the surface of hepatocytes, perhaps because of evolutionary pressure to facilitate the elimination of cells infected with hepatotropic viruses. In contrast, mitochondrial pathway is triggered by a variety of intracellular stressors such as DNA damage, growth factor deprivation, metabolic disturbances, detachment from matrix and/or surrounding cells. These two pathways are not mutually exclusive in hepatocytes, but are closely interlinked as the mitochondrial pathway is often required to amplify the relatively weak death receptor-induced apoptotic signal in all cells including hepatocytes [35].

THE ROLE OF TNF IN LIVER DISEASES

TNF is a pleiotropic pro-inflammatory cytokine produced largely by activated macrophages and in smaller amounts by several other types of cells. It exerts a variety of effects that are mediated by TNF-receptor 1 and 2 (TNFR-I and TNFR-II). The apoptotic effects are only mediated by TNFR-I, whereas TNFR-II may serve to potentiate the effects of TNFR-I in promoting cell death or promoting in-

flammation [36]. TNF was originally identified as an anti-tumor agent that induced necrotic cell death in sarcomas. However, attempts to use it for systemic anti-cancer treatment have failed due to the appearance of severe side effects [36]. One side effect is the hepatotoxicity due to the massive induction of apoptosis in hepatocytes. Subsequent studies have shown that TNF plays a role in viral hepatitis, alcoholic hepatitis, ischemia/reperfusion liver injury, and fulminant hepatic failure [37]. Serum levels of TNF are significantly increased in patients with fulminant hepatitis [37]. In viral hepatitis, elevated levels of plasma TNF and TNF-receptors are frequently observed [38].

Multiple Apoptotic Pathways Induced by TNF in Hepatocytes

TNF initiates apoptosis in hepatocytes by activating different pathways, whose subsequent activation leads to liver injury. As discussed above, the main apoptotic effects of TNF are mediated by TNFR-I. There are three functional domains within TNFR-I to transduce unique intracellular signals by interacting with different intracellular adaptor proteins [39]. They are the C-terminal death domain, the middle A-SMase (acidic sphingomyelinase) activating domain and the N-terminal N-SMase (neutral sphingomyelinase) activating domains. The death domain can mediate both the pro-apoptotic and anti-apoptotic pathways, while the other two sphingomyelinases pathways mainly modulate apoptotic and inflammatory responses [36]. These pathways have been identified to be important for TNF-induced apoptosis and liver injury, with the mitochondria acting as the central executioner for TNF-induced hepatocyte apoptosis.

The Activation of the Mitochondrial Apoptotic Pathway by TNF

Following the TNFR-I ligation, the TRADD adaptor molecule is recruited by the death domain to form the first protein complex (Complex I), which also includes TRAF2 [40]. This complex then dissociates from TNFR-I and forms a different complex in the cytosol (called Complex II). Complex II includes FADD, c-FLIP, cIAP1/2, TRAF2 and caspase-8 [40]. However, in hepatocytes, the caspase-8 complex seems rather weak in its activity, which needs to be further amplified through the mitochondrial pathway [41]. The latter is regulated by the Bcl-2 family proteins [35]. The anti-apoptotic Bcl-2 family proteins, such as Bcl-2 and Bcl-X_L, inhibit the mitochondrial death pathway, whereas pro-apoptotic Bcl-2 family proteins, such as Bid, Bax and Bak, promote it [25]. Caspase-8 can cleave Bid, a BH3-domain only Bcl-2 family protein, to form an active fragment, tBid. tBid then causes mitochondrial cristae reorganization [42] or interacts with either Bax or Bak for the release of the mitochondrial apoptotic factors, such as cytochrome c, AIF, OMI/HtrA2, and Smac/Diablo. Notably, the mitochondrial permeability transition (MPT), an important regulatory mechanism for cytochrome c release, is also induced by TNF in hepatocytes with a strong dependence on Bid [43,44]. The MPT occurs due to the opening of MPT pores, which are highly conductive to solutes with a molecular weight up to approximately 1.5 kDa [45]. As a consequence, mitochondria depolarize and the MPT can contribute to the release of apoptogenic proteins from the intermembrane space. Furthermore, activation of MPT can also lead to the generation of reactive oxygen species [46], which may in turn further enhance the MPT [45]. Suppression of MPT with cyclosporin A alone or in conjunction with its enhancer, trifluoperazine or aristolochic acid could lead to reduction of TNF-induced cytochrome c release, caspase activation and hepatocytes apoptosis [28]. More recently, it was demonstrated in a rat model that cyclosporin A prevented the hepatotoxic effects of TNF by blocking the mitochondrial pro-apoptotic pathway through inhibition of the MPT [47]. These findings on the TNF-induced mitochondria apoptotic pathway may provide a viable strategy for the treatment of liver diseases that depend on the increased production of TNF. Following cytochrome c release, a high molecular weight complex consisting of Apaf1, cytochrome c and caspase-9 is formed [48,49], which in turn activates a major execution caspase, caspase-3. Moreover, released Smac/Diablo binds to XIAP and relieves its inhibitory effect on caspase-9 and caspase-3, which therefore allow the full activation of caspase-9 or caspase-3 [50]. The above findings may explain why the mitochondrial pathway or Bid is important for TNF-induced apoptosis in hepatocytes. In addition, a Bid-independent mechanism(s) is (are) also present.

Role of Fas in Normal Liver

In humans and rats, senescent hepatocytes are eliminated via apoptosis in the acinar zone 3 of the liver [51]. However, the mechanisms of hepatocyte apoptosis under normal conditions were unknown for some time. Recently, Adachi et al. [52] generated a Fas-knockout mouse strain. In addition to a massive production of lymphocytes, the Fas-null mice showed substantial liver hyperplasia, which was accompanied by the enlargement of nuclei in hepatocytes. Apoptosis defects caused by mutations of Fas have also been found in a rare human autoimmune lymphoproliferative syndrome (ALPS) [53]. Children with ALPS are characterized by massive non-malignant lymphadenopathy, hepatosplenomegaly, autoimmunity and the presence of increased numbers of circulating and tissue TCR-αβ, CD4– CD8– T cells.

Role of Fas-Ligand in Liver Diseases

Cytotoxic T lymphocytes (CTLs) are involved in the immune clearance of hepatitis C virus (HCV) [54], or hepatitis B virus (HBV), infected hepatocytes and in the pathogenesis of these chronic viral liver diseases [55]. Short-term assays have shown that perforin and Fas ligand (FasL) are the only molecules involved in the T-cell- mediated cytodestructive effect [56]. As a result, attention has been focused on the clinical significance of Fas-mediated apoptosis in chronic hepatitis B and C. Fas is activated through oligomerization upon binding of FasL or the agonistic anti-Fas antibody. This causes formation of the death-inducing signaling complex (DISC), and the activation of downstream death signal pathway, the caspase cascade becomes activated [57]. Fas is ubiquitously expressed in various organs, including the thymus, liver, heart and kidney [58].

Hepatocellular carcinoma

Abundant cytoplasmic Fas expression has been detected in most HCC (hepatocellular carcinoma) cell lines, but only a few express Fas on their surface and tend to be sensitive to Fas stimulation [59]. Fas expression has also been exam-

ined in HCC specimens, where Fas staining was found to be less frequent and weaker in HCC tissues than in the corresponding non-cancerous tissues [60]. Furthermore, the majority of non-cancerous specimens expressed Fas both on the surface and in the cytoplasm, whereas most HCCs expressed Fas only in the cytoplasm. The number of apoptotic cells was higher in Fas-expressing tissues than in Fas-negative tissues. Among Fas expressing tissues, the extent of apoptosis was greater in surface Fas-expressing tissues than in those expressing only cytoplasmic Fas. Thus, the development of apoptosis in HCC tissues seems to be related not only to Fas expression but also to its location.

Anticancer drugs

Bleomycin, a chemotherapeutic drug, is known to induce transient accumulation of nuclear wild-type (wt) p53, which up-regulates the expression of cell surface Fas [61]. Thus, the sensitivity towards Fas mediated apoptosis was increased in wt p53-positive hepatoma cells after treatment with bleomycin. The same applies to other anticancer drugs, such as cisplatin and methotrexate. Therefore, bleomycin induced apoptosis is mediated, at least in part, by p53-dependent stimulation of the Fas system.

Alcoholic liver disease

Low constitutive levels of Fas expression, as in normal hepatocytes, have been observed in hepatocytes of alcoholic cirrhosis patients [62]. However, the hepatocytes of these patients displayed a high expression of FasL mRNA. Thus, death of Fas- and FasL-expressing hepatocytes might occur by paracrine or autocrine mechanisms. Elevation of both sFas and sFasL levels in serum was observed in acute alcoholic hepatitis and alcoholic cirrhosis [63] and might modulate hepatocyte apoptosis.

Wilson's disease

This disease can result in fulminant liver failure as a result of hepatic copper overload. In liver sections from Wilson's disease patients with fulminant liver failure, hepatocyte apoptosis was observed, characterized by an acidophilic cytoplasm, condensed chromatin and the typical rounded cell shape [64]. Furthermore, in these patients, Fas expression on hepatocyte cell membranes was heterogenously detected, and high levels of FasL transcripts were observed in areas with ongoing liver damage.

Apoptosis and Autoimmune Liver Disease

Autoimmune hepatitis and primary biliary cirrhosis (PBC) are two distinct autoimmune liver diseases. Autoimmune hepatitis is characterized by the presence of anti-nuclear antibodies or anti-LKM (Liver Kidney Microsome) antibodies and immune-mediated destruction of hepatocytes. In contrast patients with PBC generate anti-mitochondrial antibodies and initially develop immune-mediated interlobular bile duct damage. The underlying etiology of each disease remains unclear, but the mode of cell death in each disease is primarily apoptosis, presumably mediated by surrounding cytotoxic lymphocytes. Fox and colleagues noted that both FasL and granzyme B levels were increased in AIH, whereas only granzyme B expression was increased above control levels in PBC [65]. Others have not previously observed such a difference. Harada and colleagues showed that Fas expression was up regulated in damaged bile ducts of PBC [66]. FasL and Fas expression were increased on surrounding cytotoxic lymphocytes and CD68 positive cells. The development of cholestasis in PBC, as well as other cholangiopathies, may promote apoptosis induced by toxic hydrophobic bile salts such as glycodeoxycholate [67]. In contrast, ursodeoxycholate, a hydrophilic bile salt used in the treatment of PBC, reduces the number of TUNEL positive biliary duct endothelial cells (BDEC) [68]. The anti-mitochondrial antibodies seen in patients with PBC are highly specific to PBC even though the mitochondrial autoantigens are ubiquitously expressed. Interestingly, autoantibodies from patients with PBC recognize the major PBC autoantigen in apoptotic BDEC, though not in other apoptotic cell types [69]. Addition of a sulfhydryl reducing agent to non-BDEC apoptotic lysates or overexpression of Bcl-2 in non-BDEC prior to apoptosis restored or preserved autoantigen recognition. Other studies have similarly implicated apoptotic cells as sources of immunogenic forms of self-antigens in susceptible individuals [70,71]. Dalekos et al. demonstrated for the first time that in the apoptotic process, macrophage activation and the production of cytokine suppressors of haematopoiesis in bone marrow mononuclear cell from AIH-1 and PBC patients are higher than compared to controls. The Fas-FasL pathway is likely to be involved in the apoptotic process; the increased levels of selected cytokines may contribute to Fas-FasL stimulation. Cirrhosis appears unlikely to be the cause of the pathophysiologic changes described above [72].

Liver Apoptosis in Viral Hepatitis B and C

Viruses target the central parts of the proapoptotic signal transduction and execution machinery (see above and [73]). Examples of proteins that subvert pro-apoptotic signals include viral proteins that block TNF-activated signaling pathways [74] and viral proteins that inhibit ds-PKR (a protein kinase that is activated by double-stranded RNA). The ds-PKR can initiate apoptosis in virus-infected cells, viral proteins that inhibit p53 (a transcription factor that is often rate-limiting for DNA damage-induced apoptosis) [75], and viral proteins that inhibit caspases [73,76].

Hepatitis B Virus (HBV) X Protein

HBV is one of the leading causes of chronic liver disease and infection is often associated with hepatocarcinogenesis [77,78]. The X protein of HBV (HBx) is a potent transactivator essential for virus replication and shows oncogenic properties in animal models [77,78]. HBx sensitizes hepatocytes to apoptosis induced by different stimuli such TNF and TRAIL [79]. HBx is a basic protein that localizes to mitochondria, and its overexpression induces a perinuclear mitochondrial distribution coupled to $\Delta\psi_m$ loss [80]. Studies with mutant proteins reveal a MTS (mitochondria targeting sequence) in which hydrophobic residues are important for mitochondrial localization, $\Delta\psi_m$ (mitochondrial transmembrane potential) dissipation and cell death, independent of the transactivating function of HBx [81]. Moreover, PT inhibitors, antioxidants and the anti-apoptotic proteins Bcl-2 and Bcl-X_L are able to protect HBx expressing cells from death. HBx reportedly interacts with at least two mitochon-

drial proteins, namely heat shock protein 60 (Hsp60) [82] and the VDAC (voltage dependent anion channel) isoform VDAC3 [81]. It is unknown whether these interactions occur simultaneously. However, this possibility appears improbable because VDAC3 is confined to the outer mitochondrial membrane and Hsp60 is mostly located in the matrix. Interestingly, VDAC3 overexpression, mitochondrial dysfunction and changes in mitochondrial morphology have been associated with chronic liver disease and carcinogenesis [24], suggesting a pathogenic role for HBx.

HEPATITIS C VIRUS (HCV): THE NS2-PARADIGM

HCV is a RNA virus. Its RNA encodes for a polyprotein that is cleaved into the different structural and non-structural (NS) proteins [83]. HCV-NS2 is a 23-kDa hydrophobic transmembrane protein. It is localized in the endoplasmic reticulum and its function is not clearly defined. NS2 is able to bind and protect from CIDE-B-induced apoptosis. The overexpression of CIDE (cell death-inducing DFF45-like effector) proteins leads to apoptosis in many different cell lines [84]. CIDEs are localized to mitochondria and form homodimers and heterodimers with other members of the family [85,86]. Interestingly, their death-inducing activity is blocked by the overexpression of DFF-45. The C-terminal region of CIDE proteins is responsible for mitochondrial localization. NS2 from HCV interacts with CIDE-B, blocking cytochrome c release from mitochondria and cell death triggering [86]. The interaction between NS2 and CIDE-B involves the C-terminus of CIDE-B, which is responsible for dimerization, as well as a four-amino acid stretch in the NS2 protein. Double staining of NS2 and CIDE-B revealed partial overlapping signals in the perinuclear region suggesting that the NS2-CIDE-B complex may regulate apoptosis at the mitochondrial level [85]. Moreover, only those NS2 mutants that bind to CIDE-B are able to block cytochrome c release and the downstream events leading to the activation of apoptosis executioners [85].

DEVELOPMENT OF NOVEL TREATMENTS FOR LIVER DISEASES

The strict regulation of apoptotic cell death and survival pathways allows the development of therapeutic intervention strategies. Hepatocytes and stellate cells contain different protective mechanisms against cytotoxic cytokines, bile acids and reactive oxygen intermediates. Stellate cells may proliferate in response to these factors [87]. Thus, both prevention of cell death in hepatocytes and induction of apoptosis in activated stellate cells may constitute relevant therapeutic strategies. Below we highlight some of these strategies in greater detail.

Hepatocyte-directed therapy

In acute liver injury, inhibition of apoptosis of hepatocytes may be beneficial. Targets for anti-apoptotic interventions include caspases, through endogenous or exogenous caspase inhibitors, and preservation of mitochondrial integrity via anti-apoptotic Bcl-2 family members. Anti-inflammatory agents are often considered to decrease liver damage during acute liver injury; however, whether this strategy is suitable for all pathological conditions remains to be seen. For example, anti-TNF therapy in bacterial infection-induced acute liver disease prevented liver injury, but resulted in decreased bacterial clearance and decreased overall survival [88]. Some anti-inflammatory strategies may attenuate cytokine production and NF-κB activation and thus sensitize hepatocytes to apoptosis [89]. Patients who suffer from cholestatic liver injury are often treated with ursodeoxycholic acid, a bile acid, which normally constitutes 3% of total human bile acids. This substance, which was originally obtained from black bear liver, has long been used in Chinese traditional medicine for the treatment of liver diseases [90]. Recently, it has been demonstrated that the taurine conjugate of ursodeoxycholic acid protects against bile-acid induced apoptosis via direct effect on the mitochondrial membrane and via the activation of survival pathways such as mitogen-activated protein kinases (MAPKs) [87].

Therapeutic targeting of stellate cells

Acute and chronic liver injury may induce repair mechanisms, which lead to the excessive deposition of scar matrix (liver fibrosis) leading to liver cirrhosis. This is a process in which activated stellate cells are the central players. Induction of apoptotic cell death may be a promising therapeutic approach because it has recently been shown that apoptosis of activated stellate cells decreases liver fibrosis [91].

A therapeutic gene could be selectively targeted to the activated stellate cells using cell-surface markers specific for activated stellate cells, e.g. the platelet-derived growth factor (PDGF) receptor-β [92]. Target genes in stellate cells include the NF-κB, since NF-κB protects activated stellate cells against apoptotic cell death. Activation of NF-κB requires the phosphorylation of IκBα. Therefore specific delivery of an NF-κB super-repressor (a phosphorylation-resistant mutant form of IκBα) to the activated stellate cells may decrease the number of activated stellate cells and hence fibrosis [93].

Anti-apoptotic Bcl-2-family genes as a promising strategy for liver therapy

Anti-apoptotic Bcl-2 family members like Bcl-2, Bcl-X_L or A1/Bfl-1 prevent the activation of the mitochondrial/apoptosome death pathway, which is activated in hepatocytes by many noxious stimuli. Among the members of the Bcl-2 family, A1/Bfl-1, an NF-κB-regulated gene, appears to be important for hepatocyte survival. It blocks hepatocyte cell death by inhibiting the mitochondrial/apoptosome death pathway and thus the activation of the caspase cascade [89]. The protoplast of the Bcl-2 family, Bcl-2 itself, is not expressed in hepatocytes. During chronic liver injury, the expression of Bcl-2 is induced only in cholangiocytes. This implies that Bcl-2 cannot be involved in the protection of hepatocytes against bile-acid-induced liver injury. Although Bcl-2 transgenic hepatocytes are protected against Fas-induced apoptosis [94], it is not clear whether overexpression of Bcl-2 in hepatocytes will prevent necrotic cell damage resulting from chronic liver injury [95].

Caspases and their inhibitors

Besides the Bcl-2 family, caspase inhibitors, such as IAP family members, may protect against apoptotic cell death by interrupting the caspase cascade. Overexpression of the human homologue of cIAP2 did protect hepatocytes against apopto-

sis *in vitro* [46]. IAP family members selectively inhibit caspases-3 and -9. Their activity can be blocked by Smac/DIABLO and Omi/HtrA2. Therefore, the delicate balance between the relative cytosolic concentrations of active caspases-3, -8, and -9 and Smac/DIABLO, OMI/HtrA2 compared with IAP family members determine cell fate [96].

Depending on the type of injury, inhibition of certain caspases is a relevant strategy. Bajt et al. [97] have shown that, although inhibition of caspase-3 by a peptide-inhibitor inhibits LPS/GalN (d-galactosamine)- mediated apoptosis, caspase-8 inhibition is more beneficial. Furthermore, adenovirus coding for dominantnegative FADD prevented TNF/GalN-induced hepatocyte apoptosis [98]. These studies imply that, in TNF-induced apoptosis, the therapeutic intervention in the apoptotic cascade should be at the level of caspase-8. In this respect, therapy aimed at increasing the expression of c-FLIP (cellular FLICE, FADD-like IL-1β-converting enzyme inhibitory protein), the endogenous inhibitor of caspase-8, deserves further attention.

Methods to Investigate Liver Apoptosis

The methods presently used to evaluate apoptosis have some intrinsic limitations. The terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling (TUNEL) method is particularly sensitive to fixation conditions and requires highly standardized procedures. Differentiation of apoptosis from necrosis is not complete [99], and it is not easy to define the cellular origin of some nuclear reactivity, especially considering that apoptotic bodies are quickly removed by Kupffer cells, which themselves may die by apoptosis.

The classic morphologic method (H&E staining), which permits the identification of the terminal stage of hepatocellular apoptosis corresponding to the Councilman bodies, is used rarely for quantification purposes owing to the low number of appreciable events.

Many putative markers of apoptosis are under study, including enzymatic activities and neoepitopes unmasked during the apoptotic process. Among them, caspase family enzymes and cytoskeleton neoepitopes, originated as a consequence of caspase activity (for example fragments of actin and cytokeratins 18 and 19) seem particularly promising. Caspase-generated epitopes are favored by researchers because the various stimuli activating the apoptotic process, ultimately converge on the caspase system, leading to their activation [100].

One of these neoepitopes (the cleavage site on cytokeratin 18 of caspase 6), identified by a monoclonal antibody (M30), has been proposed as an apoptosis marker and was validated *in vitro* [101] and *in vivo* on trophoblast tissue in human placenta [102], endometrium [103], colon [104], and salivary glands [105]. In human liver, preliminary data have been published on primary biliary cirrhosis [106], nonalcoholic steatohepatitis [107], and hepatitis C [107]. No data is available for the sensitivity of this marker on paraffin-embedded sections compared with cryopreserved samples. Quantitative data on chronic hepatitis reported by Kronenberger et al. [108] using paraffin-embedded sections are apparently 10- to 20-fold higher than those reported by Susca et al. [107] using frozen sections. Moreover, the reported morphologic pattern of positivity is quite different: a coarse, granular, cytoplasmic positivity usually confined to a small portion of the cytoplasm in the paraffin-embedded sections, and a mainly diffuse, cytoplasmic, powder-like staining obtained on frozen sections [107], respectively.

Serum Cytochrome c Level as A New Marker for Acute Liver Damage

It has been reported recently that cytochrome c is not only released from mitochondria upon apoptosis induction, but furthermore, it can leave the apoptotic cell and thus can be detected in the extracellular fluid as an apoptosis-specific marker [46,109]. Increased cytochrome c levels have also been found in the serum of cancer patients, and its elevated level frequently correlated with the induction of chemotherapy [48,110].

Interestingly, an elevated cytochrome c level has also been observed by patients suffering from acute hepatitis. A strong correlation between the clinical symptoms of an acute liver damage and serum cytochrome c level has been described [111,112]. The serum cytochrome c level seemed to parallel the severity of hepatic coma [111]. In patients suffering from fulminant hepatitis, the serum cytochrome c level significantly correlated to serum hepatocyte growth factor, aspartate aminotransferase (AST), lactic dehydrogenase (LDH), and alkaline phosphatase (ALP), while it was negatively correlated to serum alpha-fetoprotein (AFP), and total bilirubin [111,112]. Immunohistochemical study of liver tissues obtained after transplantation indicated TdT mediated dUTP nick end-labeling (TUNEL)-positive cells in the livers of patients with fulminant hepatitis. These results suggest serum cytochrome c as a new marker for acute liver failure [111].

Epilogue

The liver is one of the largest organs in the body. Among other functions it serves as an interface that processes absorbed nutrients into chemicals that are nontoxic for the organism and can safely be utilized by other tissues and organs. It also plays an important role as a neutralizer of exo- and endotoxins. Thus, the organism cannot function without an intact liver for a prolonged time. Therefore, beside liver transplantation, several pharmacological approaches are under development that either target pathologic processes in the liver, focus on the protection of hepatocytes from noxious agents or attempt to block destructive inflammatory processes in the liver. New and old enzymatic and biochemical markers add to the vast arsenal of indicators that allow clinicians to evaluate the status of this important organ without invasive diagnostic procedures. With the increase of our understanding of the pathologic processes in the liver, and as our knowledge about diseases-induced changes in liver transcriptome and proteome advances, new drugs and new treatment modalities for acute and chronic liver disease will likely emerge in the near future.

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REFERENCES:

1. Terada T, Nakanuma Y: Detection of apoptosis and expression of apoptosis-related proteins during human intrahepatic bile duct development. Am J Pathol, 1995; 146(1): 67–74
2. Jaeschke H, Gores GJ, Cederbaum AI et al: Mechanisms of hepatotoxicity. Toxicol Sci, 2002; 65(2): 166–76
3. Johar D, Roth JC, Bay GH et al: Inflammatory response, reactive oxygen species, programmed (necrotic-like and apoptotic) cell death and cancer. Rocz Akad Med Bialymst, 2004; 4931–39
4. Lauber K, Blumenthal SG, Waibel M, Wesselborg S: Clearance of apoptotic cells: getting rid of the corpses. Mol Cell, 2004; 14(3): 277–87
5. Philchenkov A, Zavelevich M, Kroczak T J, Los M: Caspases and cancer: mechanisms of inactivation and new treatment modalities. Exp Oncol, 2004; 26(2): 82–97
6. Reed JC: Mechanisms of apoptosis. Am J Pathol, 2000; 157(5): 1415–30
7. Lemasters JJV: Necrapoptosis and the mitochondrial permeability transition: shared pathways to necrosis and apoptosis. Am J Physiol, 1999;c 276(1 Pt 1): G1–6
8. Ghavami S, Karami Tehrani F, Hashemi M et al: Possible involvement of a specific cell surface receptor for calprotectin-induced apoptosis in colon adenocarcinoma and carcinoma cell line (sw742, ht29/219). J Sci Islam Rep Iran, 2004; 15(1): 3–11
9. Panigrahi S, Mai S: Telomeres, genomic instability, DNA repair and Breast Cancer. Curr Med Chem-Anti-Inflammatory & Anti-Allergy Agents, 2005; 4(4): 421–29
10. Kreuter M et al: Stroke, myocardial infarction, acute and chronic inflammatory diseases: caspases and other apoptotic molecules as targets for drug development. Arch Immunol Ther Exp, 2004; 52(3): 141–55
11. Chlichlia K, Schirrmacher V, Sandaltzopoulos R: Cancer immunotherapy: Battling tumors with gene vaccines. Curr Med Chem-Anti-Inflammatory & Anti-Allergy Agents, 2005; 4(4): 353–67
12. Los M, Burek CJ, Stroh C et al: Anticancer drugs of tomorrow: apoptotic pathways as targets for drug design. Drug Discov Today, 2003; 8(2): 67–77
13. Mendoza FJ, Espino P, Cann CL et al: Anti-tumor chemotherapy utilizing peptide-based approaches – apoptotic pathways, kinases and proteasome as targets. Arch Immunol Ther Exp, 2005; 53, in press
14. Burek CJ, Burek M, Roth J, Los M: Calcium induces apoptosis and necrosis in hematopoetic malignant cells: Evidence for caspase-8 dependent and FADD-autonomous pathway. Gene Ther Mol Biol, 2003; 7173–79
15. Kasahara I, Saitoh K, Nakamura K: Apoptosis in acute hepatic failure: histopathological study of human liver tissue using the tunel method and immunohistochemistry. J Med Dent Sci, 2000; 47(3): 167–75
16. Gujral JS, Farhood A, Jaeschke H: Oncotic necrosis and caspase-dependent apoptosis during galactosamine-induced liver injury in rats. Toxicol Appl Pharmacol, 2003; 190(1): 37–46
17. Booy EP, Kadkhoda K, Johar D et al: The immune system, involvement in neurodegenerative diseases, ageing and cancer. Curr Med Chem-Anti-Inflammatory & Anti-Allergy Agents, 2005; 4(4): 349–53
18. Ferri KF, Kroemer G: Organelle-specific initiation of cell death pathways. Nat Cell Biol, 2001; 3(11): E255–63
19. Hashemi M, Kroczak TJ: Apoptosis and Autoimmune Disease. Curr Med Chem – Anti-Inflammatory & Anti-Allergy Agents, 2005; 4(4): 429–37
20. Los M et al: Requirement of an ICE/Ced-3 protease for Fas/Apo-1-1mediated apoptosis. Nature, 1995; 37181–83
21. Kroemer G, Dallaporta B, Resche-Rigon M: The mitochondrial death/life regulator in apoptosis and necrosis. Annu Rev Physiol, 1998; 60619–42
22. Sadowski-Debbing K, Coy JF, Mier W et al: Caspases – their role in apoptosis and other physiological processes as revealed by knock-out studies. Arch Immunol Ther Exp, 2002; 5019–34
23. Wang X: The expanding role of mitochondria in apoptosis. Genes Dev, 2001; 15(22): 2922–33
24. Waris G, Huh K W, Siddiqui A: Mitochondrially associated hepatitis B virus X protein constitutively activates transcription factors STAT-3 and NF-kappa B via oxidative stress. Mol Cell Biol, 2001; 21(22): 7721–30
25. Los M, Wesselborg S, Schulze Osthoff K: The role of caspases in development, immunity, and apoptotic signal transduction: lessons from knockout mice. Immunity, 1999; 10629–39
26. Penninger JM, Kroemer G: Mitochondria, AIF and caspases – rivaling for cell death execution. Nat Cell Biol, 2003; 5(2): 97–99
27. Susin SA et al: Molecular characterization of mitochondrial apoptosis-inducing factor. Nature, 1999; 397(6718): 441–46
28. Kroemer G: Mitochondrial control of apoptosis: an introduction. Biochem Biophys Res Commun, 2003; 304(3): 433–35
29. Zamzami N, Kroemer G: Apoptosis: mitochondrial membrane permeabilization – the (w)hole story? Curr Biol, 2003; 13(2): R71–73
30. Ghavami S, Kerkhoff C, Los M et al: Mechanism of apoptosis induced by S100A8/A9 in colon cancer cell lines: the role of ROS and the effect of metal ions. J Leukoc Biol, 2004; 76: 169–75
31. Faouzi S et al: Anti-Fas induces hepatic chemokines and promotes inflammation by an NF-kappa B-independent, caspase-3-dependent pathway. J Biol Chem, 2001; 276(52): 49: 77–82
32. Rust C, Gores GJ: Apoptosis and liver disease. Am J Med, 2000; 108(7): 567–74
33. Canbay A, Feldstein A, Baskin-Bey E et al: The caspase inhibitor IDN-6556 attenuates hepatic injury and fibrosis in the bile duct ligated mouse. J Pharmacol Exp Ther, 2004; 308(3): 1191–96
34. Canbay A, Friedman S, Gores GJ: Apoptosis: the nexus of liver injury and fibrosis. Hepatology, 2004; 39(2): 273–78
35. Yin XM, Ding WX: Death receptor activation-induced hepatocyte apoptosis and liver injury. Curr Mol Med, 2003; 3(6): 491–508
36. Wang H, Czura C, Tracey KJ, in The Cytokine Handbook, Thomson A, Lotze M, editors. Elsevier Science Ltd, London, 2003; 837–60
37. Muto Y, Nouri-Aria KT, Meager A et al: Enhanced tumour necrosis factor and interleukin-1 in fulminant hepatic failure. Lancet, 1988; 2(8602): 72–74
38. Zylberberg H et al: Soluble tumor necrosis factor receptors in chronic hepatitis C: a correlation with histological fibrosis and activity. J Hepatol, 1999; 30(2): 185–91
39. Tesarova P, Kvasnicka J, Umlaufova A et al: Soluble TNF and IL-2 receptors in patients with breast cancer. Med Sci Monit, 2000; 6(4): 661–67
40. Micheau O, Tschopp J: Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell, 2003; 114(2): 181–90
41. Pour-Jafari H, Ghavami S, Maddika S, Hashemi M: Mitochondrial Physiology and Toxicity (Mitotoxicity); Importance for the Immune System, Programmed Cell Death and Cancer. Curr Med Chem-Anti-Inflammatory & Anti-Allergy Agents, 2005; 4(4): 439–48
42. Ding WX, Ni HM, DiFrancesca D et al: Bid-dependent generation of oxygen radicals promotes death receptor activation-induced apoptosis in murine hepatocytes. Hepatology, 2004; 40(2): 403–13
43. Bradham CA, Plumpe J, Manns MP et al: Mechanisms of hepatic toxicity. I. TNF-induced liver injury. Am J Physiol, 1998; 275(3 Pt 1): G387–92
44. Zhao Y, Ding WX, Qian T et al: Bid activates multiple mitochondrial apoptotic mechanisms in primary hepatocytes after death receptor engagement. Gastroenterology, 2003; 125(3): 854–67
45. Lemasters JJ et al: The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy. Biochim Biophys Acta, 1998; 1366(1–2): 177–96
46. Schoemaker MH et al: Cytokine regulation of pro- and anti-apoptotic genes in rat hepatocytes: NF-kappaB-regulated inhibitor of apoptosis protein 2 (cIAP2) prevents apoptosis. J Hepatol, 2002; 36(6): 742–50
47. Soriano ME, Nicolosi L, Bernardi P: Desensitization of the permeability transition pore by cyclosporin a prevents activation of the mitochondrial apoptotic pathway and liver damage by tumor necrosis factor-alpha. J Biol Chem, 2004; 279(35): 36803–8
48. Barczyk K et al: Serum cytochrome c indicates *in vivo*-apoptosis and it can serve as a prognostic marker during cancer therapy. In J Cancer, 2005; 116(2): 167–73
49. Jiang X, Wang X: Cytochrome c promotes caspase-9 activation by inducing nucleotide binding to Apaf-1. J Biol Chem, 2000; 275(40): 31199–203
50. Li S et al: Relief of extrinsic pathway inhibition by the Bid-dependent mitochondrial release of Smac in Fas-mediated hepatocyte apoptosis. J Biol Chem, 2002; 277(30): 26912–20
51. Hayashi N, Mita E: Involvement of Fas system-mediated apoptosis in pathogenesis of viral hepatitis. J Viral Hepat, 1999; 6(5): 357–65
52. Adachi M et al: Targeted mutation in the Fas gene causes hyperplasia in peripheral lymphoid organs and liver. Nat Genet, 1995; 11(3): 294–300
53. Fisher GH et al: Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. Cell, 1995; 81(6): 935–46
54. Gabriel A, Ziolkowski A, Dziambor AP et al:.Bcl-2 antigen expression in patients with chronic hepatitis C and advanced fibrosis. Med Sci Monit, 2001; 7(Suppl.1): 181–84

55. Chisari FV: Cytotoxic T cells and viral hepatitis. J Clin Invest, 1997; 99(7): 1472–77
56. Kagi D et al: Fas and perforin pathways as major mechanisms of T cell-mediated cytotoxicity. Science, 1994; 265(5171): 528–30
57. Depraetere V, Golstein P: Fas and other cell death signaling pathways. Semin Immunol, 1997; 9(2): 93–107
58. Watanabe-Fukunaga R et al: The cDNA structure, expression, and chromosomal assignment of the mouse Fas antigen. J Immunol, 1992; 148(4): 1274–79
59. Natoli G et al: Resistance to Fas-mediated apoptosis in human hepatoma cells. Oncogene, 1995; 11(6): 1157–64
60. Higaki K, Yano H, Kojiro M: Fas antigen expression and its relationship with apoptosis in human hepatocellular carcinoma and noncancerous tissues. Am J Pathol, 1996; 149(2): 429–37
61. Muller M et al: Drug-induced apoptosis in hepatoma cells is mediated by the CD95 (APO-1/Fas) receptor/ligand system and involves activation of wild-type p53. J Clin Invest, 1997; 99(3): 403–13
62. Galle PR et al: Involvement of the CD95 (APO-1/Fas) receptor and ligand in liver damage. J Exp Med, 1995; 182(5): 1223–30
63. Taieb J, Mathurin P, Poynard T et al: Raised plasma soluble Fas and Fas-ligand in alcoholic liver disease. Lancet, 1998; 351(9120): 1930–31
64. Strand S et al: Hepatic failure and liver cell damage in acute Wilson's disease involve CD95 (APO-1/Fas) mediated apoptosis. Nat Med, 1998; 4(5): 588–93
65. Fox CK, Furtwaengler A, Nepomuceno RR et al: Apoptotic pathways in primary biliary cirrhosis and autoimmune hepatitis. Liver, 2001; 21(4): 272–79
66. Harada K, Ozaki S, Gershwin ME, Nakanuma Y: Enhanced apoptosis relates to bile duct loss in primary biliary cirrhosis. Hepatology, 1997; 26(6): 1399–405
67. Higuchi H et al: The bile acid glycochenodeoxycholate induces trail-receptor 2/DR5 expression and apoptosis. J Biol Chem, 2001; 276(42): 38610–18
68. Botla R, Spivey JR, Aguilar H et al: Ursodeoxycholate (UDCA) inhibits the mitochondrial membrane permeability transition induced by glycochenodeoxycholate: a mechanism of UDCA cytoprotection. J Pharmacol Exp Ther, 1995; 272(2): 930–38
69. Odin JA, Huebert RC, Casciola-Rosen L et al: Bcl-2-dependent oxidation of pyruvate dehydrogenase-E2, a primary biliary cirrhosis autoantigen, during apoptosis. J Clin Invest, 2001; 108(2): 223–32
70. Matsumura S et al: Contribution to antimitochondrial antibody production: cleavage of pyruvate dehydrogenase complex-E2 by apoptosis-related proteases. Hepatology, 2002; 35(1): 14–22
71. Rosen A, Casciola-Rosen L, Ahearn J: Novel packages of viral and self-antigens are generated during apoptosis. J Exp Med, 1995; 181(4): 1557–61
72. Tsikrikoni A et al: Markers of cell activation and apoptosis in bone marrow mononuclear cells of patients with autoimmune hepatitis type 1 and primary biliary cirrhosis. J Hepatol, 2005; 42(3): 393–99
73. Cassens U et al: Viral modulation of cell death by inhibition of caspases. Arch Immunol Ther Exp, 2003; 51(1): 19–27
74. Hay S, Kannourakis G: A time to kill: viral manipulation of the cell death program. J Gen Virol, 2002; 83(Pt 7): 1547–64
75. Finlan LE, Hupp TR: The Life Cycle of p53: a Key Target in Drug Development, in Apoptotic pathways as target for novel therapies in cancer and other diseases, Los M, Gibson SB, Editors. Kluwer Academic Press, New York, 2005
76. Nogal ML et al: African swine fever virus IAP homologue inhibits caspase activation and promotes cell survival in mammalian cells. J Virol, 2001; 75(6): 2535–43
77. Kim CM, Koike K, Saito I et al: HBx gene of hepatitis B virus induces liver cancer in transgenic mice. Nature, 1991; 351(6324): 317–20
78. Wang Y, Lau SH, Sham JS et al: Characterization of HBV integrants in 14 hepatocellular carcinomas: association of truncated X gene and hepatocellular carcinogenesis. Oncogene, 2004; 23(1): 142–48
79. Su F, Schneider RJ: Hepatitis B virus HBx protein sensitizes cells to apoptotic killing by tumor necrosis factor alpha. Proc Natl Acad Sci USA, 1997; 94(16): 8744–49
80. Henkler F et al: Intracellular localization of the hepatitis B virus HBx protein. J Gen Virol, 2001; 82(Pt 4): 871–82
81. Rahmani Z, Huh KW, Lasher R, Siddiqui A: Hepatitis B virus X protein colocalizes to mitochondria with a human voltage-dependent anion channel, HVDAC3, and alters its transmembrane potential. J Virol, 2000; 74(6): 2840–46
82. Tanaka Y et al: Interaction of the hepatitis B virus X protein (HBx) with heat shock protein 60 enhances HBx-mediated apoptosis. Biochem Biophys Res Commun, 2004; 318(2): 461–69
83. Giannini C, Brechot C: Hepatitis C virus biology. Cell Death Differ, 2003; 10(Suppl.1): S27–38
84. Inohara N, Koseki T, Chen S et al: CIDE, a novel family of cell death activators with homology to the 45 kDa subunit of the DNA fragmentation factor. Embo J, 1998; 17(9): 2526–33
85. Erdtmann L et al: The hepatitis C virus NS2 protein is an inhibitor of CIDE-B-induced apoptosis. J Biol Chem, 2003; 278(20): 18256–64
86. Franck N, Le Seyec J, Guguen-Guillouzo C, Erdtmann L: Hepatitis C virus NS2 protein is phosphorylated by the protein kinase CK2 and targeted for degradation to the proteasome. J Virol, 2005; 79(5): 2700–8
87. Schoemaker MH, Moshage H: Defying death: the hepatocyte's survival kit. Clin Sci (Lond), 2004; 107(1): 13–25
88. Moore TA, Lau HY, Cogen AL et al: Anti-tumor necrosis factor-alpha therapy during murine Klebsiella pneumoniae bacteremia: increased mortality in the absence of liver injury. Shock, 2003; 20(4): 309–15
89. Schoemaker MH et al: Tauroursodeoxycholic acid protects rat hepatocytes from bile acid-induced apoptosis via activation of survival pathways. Hepatology, 2004; 39(6): 1563–73
90. Paumgartner G, Beuers U: Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. Hepatology, 2002; 36(3): 525–31
91. Wright M C et al: Gliotoxin stimulates the apoptosis of human and rat hepatic stellate cells and enhances the resolution of liver fibrosis in rats. Gastroenterology, 2001; 121(3): 685–98
92. Beljaars L, Weert B, Geerts A et al: The preferential homing of a platelet derived growth factor receptor-recognizing macromolecule to fibroblast-like cells in fibrotic tissue. Biochem Pharmacol, 2003; 66(7): 1307–17
93. Lang A, Schoonhoven R, Tuvia S et al: Nuclear factor kappaB in proliferation, activation, and apoptosis in rat hepatic stellate cells. J Hepatol, 2000; 33(1): 49–58
94. Mignon A et al: Selective repopulation of normal mouse liver by Fas/CD95-resistant hepatocytes. Nat Med, 1998; 4(10): 1185–88
95. Kane DJ, Ord T, Anton R, Bredesen DE: Expression of bcl-2 inhibits necrotic neural cell death. J Neurosci Res, 1995; 40(2): 269–75
96. Li F, Survivin, other IAPs, Smac/DIABLO, and OMI/HtrA2 – Modulation of the Advancing Apoptotic Process, in Apoptotic pathways as target for novel therapies in cancer and other diseases, Los M, Gibson SB, Editors, Springer Science + Business Media, Inc., New York, 2005
97. Bajt ML, Vonderfecht SL, Jaeschke H: Differential protection with inhibitors of caspase-8 and caspase-3 in murine models of tumor necrosis factor and Fas receptor-mediated hepatocellular apoptosis. Toxicol Appl Pharmacol, 2001; 175(3): 243–52
98. Streetz K et al: Tumor necrosis factor alpha in the pathogenesis of human and murine fulminant hepatic failure. Gastroenterology, 2000; 119(2): 446–60
99. Charriaut-Marlangue C, Ben-Ari Y: A cautionary note on the use of the TUNEL stain to determine apoptosis. Neuroreport, 1995; 7(1): 61–64
100. Kumar S: Regulation of caspase activation in apoptosis: implications in pathogenesis and treatment of disease. Clin Exp Pharmacol Physiol, 1999; 26(4): 295–303
101. Leers M P et al: Immunocytochemical detection and mapping of a cytokeratin 18 neo-epitope exposed during early apoptosis. J Pathol, 1999; 187(5): 567–72
102. Chiu PM, Ngan YS, Khoo US, Cheung AN: Apoptotic activity in gestational trophoblastic disease correlates with clinical outcome: assessment by the caspase-related M30 CytoDeath antibody. Histopathology, 2001; 38(3): 243–49
103. Morsi HM et al: The patterns of expression of an apoptosis-related CK18 neoepitope, the bcl-2 proto-oncogene, and the Ki67 proliferation marker in normal, hyperplastic, and malignant endometrium. Int J Gynecol Pathol, 2000; 19(2): 118–26
104. Backus HH et al: Differential expression of cell cycle and apoptosis related proteins in colorectal mucosa, primary colon tumours, and liver metastases. J Clin Pathol, 2002; 55(3): 206–11
105. Kusama K et al: Use of the monoclonal antibody M30 for detecting HSG cell apoptosis. Anticancer Res, 2000; 20(1A): 151–54
106. Ballardini G et al: Bile duct cell apoptosis is a rare event in primary biliary cirrhosis. Dig Liver Dis, 2001; 33(2): 151–56
107. Susca M et al: Liver inflammatory cells, apoptosis, regeneration and stellate cell activation in non-alcoholic steatohepatitis. Dig Liver Dis, 2001; 33(9): 768–77

108. Kronenberger B et al: Hepatocellular proliferation in patients with chronic hepatitis C and persistently normal or abnormal aminotransferase levels. J Hepatol, 2000; 33(4): 640–47

109. Ahlemeyer B, Klumpp S, Krieglstein J: Release of cytochrome c into the extracellular space contributes to neuronal apoptosis induced by staurosporine. Brain Res, 2002; 934(2): 107–16

110. Renz A, Berdel WE, Kreuter M et al: Rapid extracellular release of cytochrome c is specific for apoptosis and marks cell death *in vivo*. Blood, 2001; 98: 1542–48

111. Sakaida I et al: Cytochrome c is a possible new marker for fulminant hepatitis in humans. J Gastroenterol, 2005; 40(2): 179–85

112. Ben-Ari Z et al: Circulating soluble cytochrome c in liver disease as a marker of apoptosis. J Intern Med, 2003; 254(2): 168–75