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Apoptosis and oncotic necrosis: profibrotic signalling mechanisms of cell death

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INTRODUCTION

Liver fibrosis forms part of most chronic liver disease processes including alcoholic and non-alcoholic steatohepatitis, viral and autoimmune hepatitis and obstructive cholestasis[1]. Key features of fibrogenesis include activation of stellate cells and the deposition of excess extracellular matrix, especially the fibril-forming collagens I and III, in the space of Disse[1,2]. These events can lead to a significant impairment of liver function and portal hypertension, and can enhance susceptibility to sepsis. The fibrotic response is initiated and maintained by acute and chronic cell injury. The fibrotic process can be stopped and even reversed by removing the chronic insult, by inducing cell death of activated stellate cells and by promoting degradation of the excess extracellular matrix. Thus, controlling cell death and its profibrotic signalling events are critical therapeutic strategies to prevent or reverse fibrosis.

APOPTOTIC CELL DEATH

During the past decade major advances have been made in the understanding of signalling mechanisms of apoptotic cell death in hepatocytes and other cell types[3–6]. Depending on the initiating signal, two types of apoptosis can be distinguished. In the extrinsic pathway (Figure 1), a ligand, e.g. Fas ligand, binds to its respective death receptor, i.e. Fas receptor. Ligand binding results in trimerization of the receptor, which allows the recruitment of adapter molecules and proenzymes of initiator caspases to form the death-inducing signalling complex (DISC). Assembly of the DISC leads to formation of the active caspase-8 or -10, which cleave the Bcl-2 family member Bid. The truncated form of Bid (tBid) triggers the translocation of Bax to the outer membrane of the mitochondria, where Bax together with other pro-apoptotic Bcl-2 family members (Bak, Bad) form pores in the outer membrane and

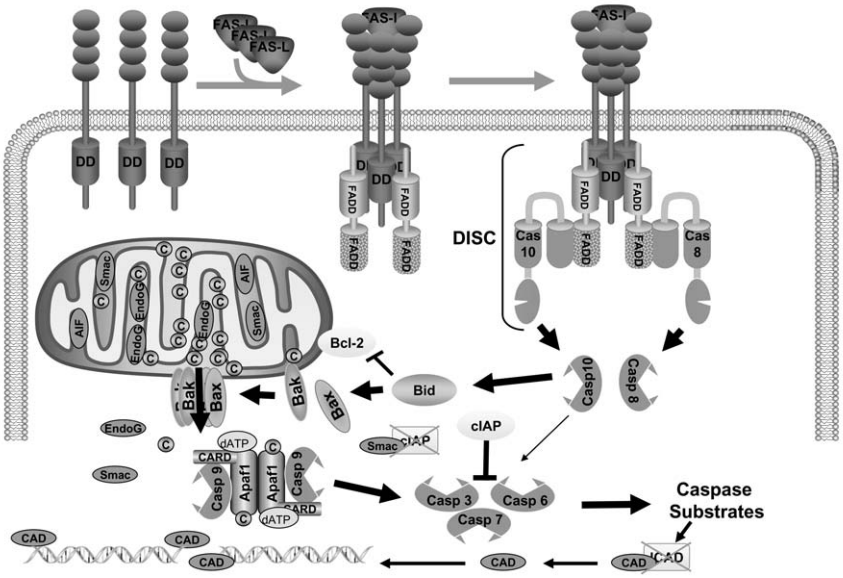

Figure 1 Fas receptor-mediated (extrinsic) pathway of apoptotic signalling in hepatocytes. See text for details. AIF, apoptosis-inducing factor; Apaf1, apoptosis protease-activating factor-1; CAD, caspase-activated DNase; CARD, caspase-activating and -recruiting domain; Casp, caspase; c, cytochrome c; cIAP, cellular inhibitor of apoptosis proteins; DD, death domain; Smac, second mitochondria-derived activator of caspases; DISC, death-inducing signalling complex; EndoG, endonuclease G; FADD, Fas-associated death domain; FAS-L, Fas-ligand; ICAD, inhibitor of CAD. Adapted from ref. 4. Reproduced with permission

release intermembrane proteins such as cytochrome c, second mitochondria-derived activator of caspases (Smac), apoptosis-inducing factor (AIF) and endonuclease G. An increase of cytochrome c levels in the cytosol leads to the assembly of the apoptosome, which triggers the autocatalytic processing of procaspase-9 to the active enzyme. Caspase-9 processes procaspase-3, the major effector caspase of apoptosis. Hepatocytes also contain inhibitors of apoptosis proteins (IAP), which bind to active caspases and prevent the accidental propagation of the apoptotic signalling cascade. In order to overcome this safety measure within the cell, Smac released from mitochondria binds to IAP and promotes their proteolytic degradation, thereby allowing the uninhibited activation of the caspase cascade. Caspase-3 and other downstream effector caspases such as caspase-6 and -7 cleave numerous substrates within the cell, resulting in the characteristic morphological changes of apoptotic cells[7]. These morphological changes include cell shrinkage, membrane blebbing, chromatin condensation and margination along the nuclear envelope, DNA fragmentation and ultimately formation of apoptotic bodies, which are internalized by neighbouring cells or phagocytosed by hepatic macrophages (Kupffer cells).

In contrast to the extrinsic, receptor-mediated signalling mechanism, the intrinsic pathway is initiated by an internal insult[4,8]. For example, DNA damage can lead to p53 activation and the increased formation of Bax. Alternatively, disturbances of the cellular Ca^{2+} homeostasis can trigger the cleavage of Bid by activation of calpains. These internal initiating events induce the release of cytochrome c from mitochondria and all subsequent signalling mechanisms described previously. In general, if a limited number of cells undergo apoptosis due to a moderate stress, the process can be completed, i.e. the apoptotic bodies can be eliminated without triggering an inflammatory response. However, if the pro-apoptotic stimulus is too severe, and affects many cells, mitochondria undergo the membrane permeability transition pore opening, which causes the collapse of their membrane potential and results in declining cellular adenosine triphosphate (ATP) levels[3,9]. Under these conditions the apoptotic process deteriorates into secondary necrosis with release of cell contents[10]. The important feature of secondary necrosis is that many characteristics of apoptosis, e.g. caspase activation and nuclear changes, are still maintained[11].

ONCOTIC NECROSIS

In the past, oncotic necrosis was considered the consequence of a catastrophic event, which resulted in immediate cell death. Although such a massive insult may occur under special circumstances, the more likely scenario is that a moderate external interference with the cellular homeostasis can induce disturbances within the cell, resulting in ultimate cell death. The external insult triggers a necrotic signalling cascade, which may have certain overlap with apoptotic signalling but is clearly a separate process. An example of this necrotic signalling is liver injury induced by acetaminophen (AAP) overdose (Figure 2)[12]. The injury process is initiated by the formation of a reactive metabolite (*N*-acetyl-*p*-benzoquinone imine; NAPQI) through the P450 system. NAPQI can be detoxified by conjugation with glutathione. However, once the cellular glutathione pool is exhausted, excess NAPQI can bind to cellular proteins including mitochondrial proteins. This early cellular stress also causes activation of Bid[12] and c-jun N-terminal kinase[13], which can promote the translocation of Bax to the outer mitochondrial membrane[14]. The mitochondrial translocation of Bax promotes the release of intermembrane proteins. Although cytochrome c and Smac are released from mitochondria after AAP overdose[14], there is no relevant caspase activation, mainly due to the declining cellular ATP levels[15]. On the other hand, mitochondrial release of AIF and endonuclease G results in translocation of these proteins to the nucleus where they induce DNA fragmentation[16,17]. In addition to these signalling events the early mitochondrial dysfunction also results in formation of reactive oxygen species and peroxynitrite[12]. The oxidant stress is mainly responsible for the opening of the mitochondrial membrane permeability transition pore, which leads to the collapse of the mitochondrial membrane potential, mitochondrial swelling and rupture of the outer membrane[18]. At this point mitochondrial release of AIF and endonuclease G and the resulting DNA

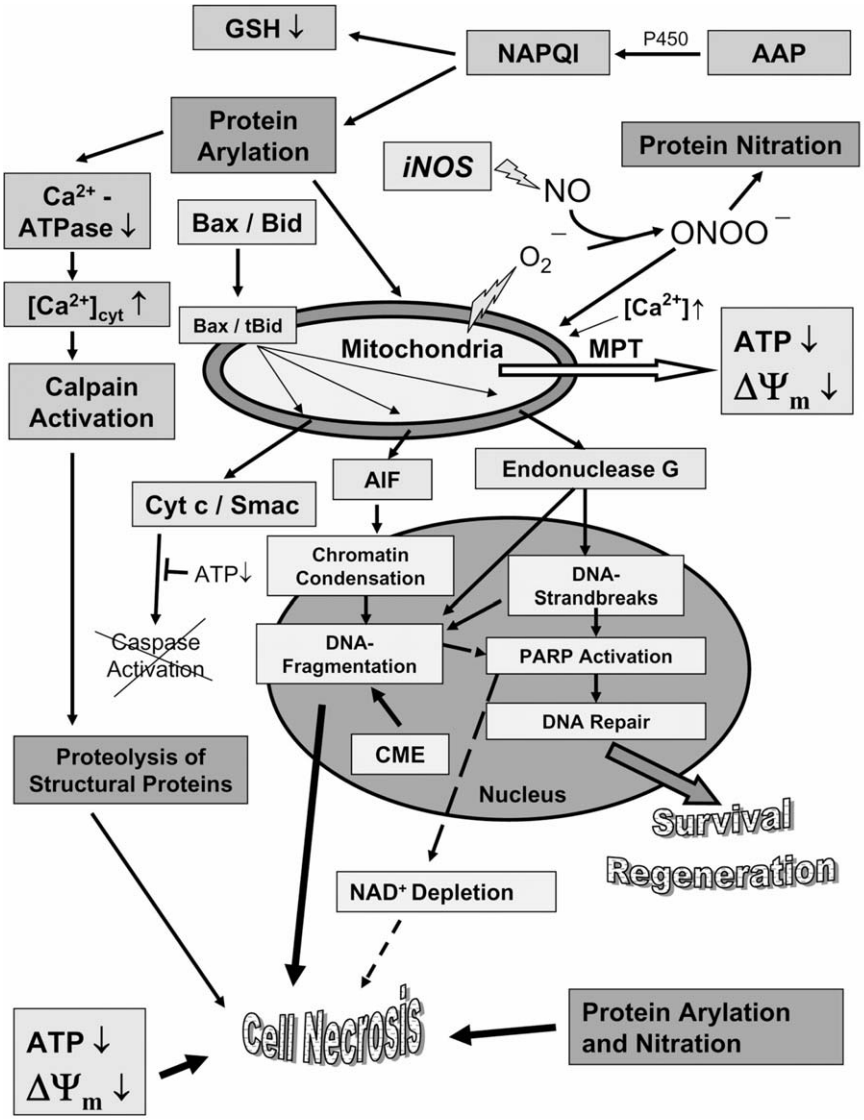

Figure 2 Intracellular signalling mechanisms of oncotic necrosis induced by acetaminophen (AAP) overdose. See text for details. AIF, apoptosis-inducing factor; CMA, nuclear Ca^{2+}/Mg^{2+}-dependent endonuclease; cyt c, cytochrome c; $\Delta\Psi_m$, mitochondrial membrane potential; GSH, reduced glutathione; iNOS, inducible NO synthase; MPT, mitochondrial membrane permeability transition; NAPQI, *N*-acetyl-*p*-benzoquinone imine; NO, nitric oxide; O_2, superoxide; $ONOO^-$, peroxynitrite; PARP, poly(ADP-ribose) polymerase; Smac, second mitochondria-derived activator of caspases; tBid, truncated form of Bid. Adapted from ref. 12. Reproduced with permission from Oxford University Press

fragmentation is independent of Bax and other Bcl-2 family members[14]. The extensive mitochondrial dysfunction with cessation of ATP production and endonuclease-mediated nuclear DNA fragmentation leads to oncotic necrosis cell death with cell swelling and release of cellular contents (Figure 2). In general, oncotic necrosis in hepatocytes can be induced by a variety of insults including ischaemia–reperfusion, bile acids, and various drugs and chemicals. Although the initiating events may vary, reactive metabolite formation, oxidative stress and mitochondrial dysfunction are common features of many pathophysiological processes[19]. In addition, the intracellular signalling events leading to cell death may be enhanced by triggering an inflammatory response, which can substantially enhance the oxidant stress and aggravate the existing injury[20]. However, the critical message is that not only apoptosis but also oncotic necrosis can be pharmacologically manipulated after the initial insults.

PATHOPHYSIOLOGICAL IMPORTANCE OF APOPTOSIS VERSUS ONCOTIC NECROSIS

Because of overlapping signalling pathways, and the fact that many parameters which investigators use to quantify cell death are not specific for apoptosis or oncotic necrosis, there is a substantial controversy regarding the predominant mode of cell death in various pathophysiologies[3,5,11]. However, it is important to remember that apoptosis is defined by morphology (cell shrinkage, chromatin condensation, formation of apoptotic bodies) and that the activation of executioner caspases is mainly responsible for these characteristic morphological features[7]. Thus, the conclusion that cell death is caused by apoptosis needs to be based on these morphological features together with quantitatively relevant activation of caspases[3,5,11]. Many other parameters including DNA fragmentation (TUNEL assay, DNA ladder, anti-histone ELISA), mitochondrial Bax and Bid translocation, Bid cleavage, mitochondrial cytochrome c release, increased Fas receptor expression, etc., are not specific for apoptosis. These parameters are only useful to define signalling mechanisms once, based on morphological evidence, the mode of cell death has been determined. Although it may seem to be sometimes a futile argument whether to label the cell death as apoptosis or oncotic necrosis, it actually is an important distinction. To resolve an inflammatory or fibrotic process it is necessary to promote apoptosis of activated neutrophils or stellate cells, respectively[21,22]. In this respect it is critical to define whether the chronic cell injury promoting inflammation or fibrosis is due to apoptosis or oncotic necrosis. It would be more challenging to identify therapeutic targets if apoptotic cell death is required for both the promotion of fibrosis and its resolution.

PROFIBROTIC SIGNALLING DURING CELL DEATH

Chronic cell injury and stellate cell activation are critical features of liver fibrogenesis[2]; however, the molecular connection between these events became

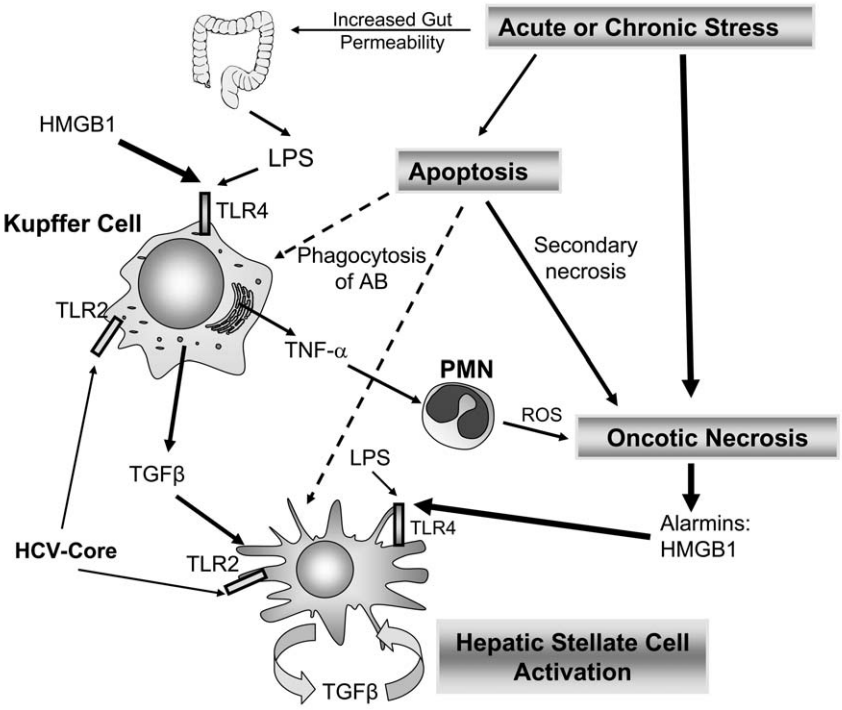

Figure 3 Profibrotic signalling mechanisms of cell death. See text for details. AB, apoptotic bodies; HCV-core, hepatitis C virus core protein; HMGB1, high-mobility group box 1 protein; LPS, lipopolysaccharide, endotoxin; PMN, polymorphonuclear leukocytes; ROS, reactive oxygen species; TGF-β, transforming growth factor β; TLR, toll-like receptor; TNF-α, tumour necrosis factor-α

evident only recently. In the case of apoptotic cell death the main products are apoptotic bodies, which are phagocytosed by macrophages (Kupffer cells) and to a lesser degree also by stellate cells through interaction with the phosphatidyl serine receptor[23,24]. In Kupffer cells the phagocytosis of apoptotic bodies leads to proinflammatory cytokine expression, e.g. tumour necrosis factor alpha (TNF-α), but not to formation of the profibrogenic cytokine-transforming growth factor-β_1 (TGF-β_1)[25]. In contrast, phagocytosis of apoptotic bodies in stellate cells caused the formation of TGF-β_1 and collagen α_1[24]. Thus, extensive apoptotic cell death triggers stellate activation either directly or indirectly through Kupffer cells, and can promote hepatic fibrosis[24,25].

A hallmark of oncotic necrosis is the release of cell contents including a number of proteins generally termed alarmins[26]. High-mobility group box-1 protein (HMGB1), a nuclear protein, is selectively released by necrotic cells[27]. Interestingly, HMGB1 and other alarmins are recognized by toll-like receptors (TLR) on Kupffer cells and stellate cells[28,29]. Signalling through TLR4, which recognizes HMGB1 and endotoxin, triggers proinflammatory and

profibrogenic cytokine formation in Kupffer cells and in stellate cells[30]. Thus, Kupffer cell activation by alarmins and/or phagocytosis of necrotic cell debris contribute to fibrogenesis directly by release of TGF-β, which activates stellate cells, and indirectly by further promoting inflammation (leukocyte infiltration) and cell injury (reactive oxygen and cytokine formation) (Figure 3). Oxidant stress generated extracellularly by inflammatory cells or intracellularly does not induce apoptosis, but causes cell death mainly through oncotic necrosis[31,32] and promotes fibrosis through alarmin signalling. In addition, during viral hepatitis, viral proteins may directly contribute to fibrogenesis through their recognition by the TLR2 receptor on Kupffer and stellate cells[28,29]. In cases where the permeability of the intestinal wall is increased, e.g. ethanol toxicity, or the gut flora is changed, e.g. obstructive cholestasis, endotoxin plays an important role in enhancing inflammatory cell injury and fibrosis[30,33].

SUMMARY

Both apoptosis and oncotic necrosis can indirectly cause stellate cell activation through mediators generated by activated Kupffer cells, which release proinflammatory and profibrotic cytokines in response to TLR signalling induced by alarmins, viral proteins and endotoxin, as well as phagocytosis of apoptotic bodies and necrotic cell debris. Since stellate cells express the same TLR as Kupffer cells, and are able to recognize and phagocytose apoptotic bodies, the same mediators can also directly activate stellate cells and contribute to their continued activation. On the other hand, resolution of inflammation and fibrogenesis depends on the removal of activated leukocytes and stellate cells by apoptosis. Therefore, it is an opportunity, but also a challenge, to selectively inhibit the chronic, profibrotic cell death signalling but induce, or at least not interfere with, apoptosis of stellate cells and leukocytes. A better understanding of the intracellular signalling mechanisms of cell death induced by various insults remains an important goal for the future.

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