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Review

Molecular mechanisms of liver injury: Apoptosis or necrosis

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ABSTRACT

Hepatic apoptosis is thought of as a prevalent mechanism in most forms of liver injury. However, the role of hepatic apoptosis is often intermixed with the cellular necrosis. It remains unknown how apoptosis is relevant to the progression of the liver injury. This review summarizes the characteristics of both hepatic apoptosis and necrosis in pathogenesis of liver diseases. Apoptosis and necrosis represent alternative outcomes of different etiology during liver injury. Apoptosis is a main mode of cell death in chronic viral hepatitis, but is intermingled with necrosis in cholestatic livers. Necrosis is the principal type of liver cell killing in acetaminophen-induced hepatotoxicity. Anti-apoptosis as a strategy is beneficial to liver repair response. Therapeutic options of liver disease depend on the understanding toward pathogenic mechanisms of different etiology.

Contents

1. Pathogenic mechanisms of liver diseases

Liver disease can be induced by different factors such as viruses, alcohol, toxic bile acids, fatty acids, drugs, and immune response (Sharma, 2013; Younossi et al., 2013; Halilbasic et al., 2013; Bechmann et al., 2013; Ghabril et al., 2013; Invernizzi, 2011). The pre-existing genetic condition modifies susceptibility to various types of causative factors and perpetuates the destruction of liver tissue (Dongiovanni et al., 2013). In the injured liver, cell death modes include necrosis, necroptosis, autophagy, and apoptosis (Roychowdhury et al., 2013). These diverse modes of cell death are intermingled as a continuous process. They consist in a dynamic spectrum during liver injury. Apoptosis and necrosis are two major types of cell death (Shuh et al., 2013). Apoptosis is an early, chronic, and temperate response subsequent to injury-initiation, whereas

Abbreviations: ATP, adenosine triphosphate; DNA, deoxyribonucleic acid; AIF, apoptosis-inducing factor; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling; ELISA, enzyme-linked immunosorbent assay; TNFα, tumor necrosis factor-alpha; TNFR, tumor necrosis factor receptor; TRAIL, TNF-related apoptosis-inducing ligand; FADD, Fas-Associated protein with Death Domain; TRADD, tumor necrosis factor receptor type 1-associated death domain protein; TGFβ, transforming growth factor beta; IFN-γ, interferon-gamma; IL, interleukin; CCL3, chemokine (C-C motif) ligand 3; CCL4, chemokine (C-C motif) ligand 4; CXCR3, chemokine (C-X-C motif) receptor 3; IP-10, interferon (IFN)-gamma inducible protein 10; CCR5, chemokine (C-C motif) receptor 5; RANTES, regulated on activation normal T cell expressed and secreted; CD95, cluster of differentiation 95; Bax, Bcl2-associated X protein; Bad, Bcl2-associated death promoter protein; Bid, BH3 interacting-domain death agonist; APAP, N-acetyl-p-aminophenol or acetaminophen; AP-1, activator protein 1.

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necrosis is an acute and severe reply. Causative factors of liver disease may induce both modes of cell death dependent on the severity of the insult. A central but controversial issue in the debate on the mechanisms of cell death during liver diseases is whether the liver injury results from apoptosis or necrosis. Which mode (apoptosis or necrosis) is main mechanism? This is a key point that makes therapeutic options into quite different directions depending on the answer. Apoptosis is associated with little secondary impairment as compared with necrosis. The necrotic process recruits inflammatory cells (e.g. neutrophils) into liver parenchyma. Liver injury is further aggravated by inflammatory response, which leads to dramatically different outcome from apoptotic cell death in regard to progression of liver disease (Sanz-Garcia et al., 2013).

2. Comparison between apoptosis and necrosis

Apoptosis is programmed cell death or to commit suicide, but necrosis is a premature death of cells. Apoptosis covers physiological response (e.g. to remove aged cells, follicular atresia of the postovulatory follicle and post-weaning mammary gland involution) and pathological conditions (Ozawa, 1995; Tilly et al., 1991; Lund et al., 1996), whereas necrosis is a pathologic process that is often caused by external factors (e.g. infection, toxins, and trauma) to the cell or tissue (Barnes et al., 2013). Characteristics of apoptosis and necrosis are distinctly identified. In morphology, apoptotic features include alterations in membrane asymmetry, budding of plasma membrane without loss of integrity, mitochondrial permeability transition due to pore formation, shrinking of cytoplasm, aggregation of chromatin at the nuclear membrane, condensation of nucleus, formation of membrane bound vesicles, and fragmentation of cell into smaller bodies (apoptotic bodies) (Elmore, 2007). Necrosis shows blebbing of membrane integrity, swelling of cytoplasm, mitochondrial swelling and calcification, disintegration of organelles, and cell lysis (Trump et al., 1997). In biochemistry, apoptotic events contain translocation of phosphatidylserine from the cytoplasmic to the extracellular side of the membrane, tightly regulated enzymatic process and activation of caspase cascade, release of mitochondrial factors (cytochrome *c*, AIF) into cytoplasm, an energy (ATP)-dependent and active process, and mono- and oligonucleosomal length fragmentation of DNA (Elmore, 2007). Necrotic features have no energy requirement, loss of regulation of ion homeostasis, random digestion of DNA, and postlytic DNA fragmentation. Apoptosis is a controlled death. The apoptotic cell breaks down into smaller fragments (or apoptotic bodies). These fragments are enclosed in membranes so as not to harm nearby cells. Adjacent cells or macrophages (or Kupffer cells in liver) engulf and destroy the apoptotic bodies (Canbay et al., 2003a). However, inner cell components are splashed out during necrosis, which elicits neutrophil invasion and a significant inflammatory response (Shi et al., 1996).

3. Hepatic apoptosis

Although both apoptosis and necrosis are basic mechanisms for the pathogenesis of liver injury, the apoptosis is thought of as a common mode of cell death in chronic liver diseases (Hikita et al., 2011). Hepatic apoptosis, as a prominent pathological feature of chronic liver injury, determines the progression of liver disease (Mundt et al., 2005). Hepatocyte apoptosis is characterized by ATP-dependent biochemical mechanisms and apparent morphological changes such as nuclear chromatin condensation, chromosomal DNA fragmentation, cell shrinkage, and membrane budding (Elmore, 2007). Apoptosis shares general machinery for cell death, including death receptor-dependent (extrinsic) pathway and mitochondrial dependent (intrinsic) pathway (Hikita et al., 2011; Guha et al., 2007). Apoptotic bodies are phagocytosed by Kupffer cells in liver (Canbay et al., 2003a). Apoptosis is associated with multiple pathophysiological functions. Dysfunction or dysregulation of the apoptotic program is implicated in a variety of congenital anomalies, tumorigenesis and autoimmune diseases (Torchinsky et al., 1995; Wahl et al., 2013; Mason et al., 2013). Owing to complicate etiology of liver injury, hepatic apoptosis and its pathophysiological role have much discrepancy as well. The hepatic apoptosis can be modulated by different etiology, distinct mechanisms and diverse regulation.

4. Detection of apoptosis

Apoptosis is characterized by membrane asymmetry, cellular shrinking, caspase activation, DNA fragmentation, chromatin condensation, and no release of cellular contents (Elmore, 2007). Apoptotic cell death can be identified through particular morphology in single cell by electron microscopy (Zhao et al., 2012), externalization of phosphatidylserine via flow cytometry (Tan et al., 2013), DNA fragmentation as detected by DNA laddering (Wang et al., 2005), terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay, histone ELISA (Cai et al., 2013), mitochondrial cytochrome *c* release (Hikita et al., 2011), translocation of the pro-apoptotic Bcl-2 family member Bax to the mitochondria (Ola et al., 2011) and the hepatoprotective effect of the pancaspase inhibitor following pretreatment (Gujral et al., 2004). Apoptosis is rather low in healthy liver and less than 0.5% of apoptotic hepatocytes are seen in viral hepatitis (Calabrese et al., 2000). DNA fragmentation assay and caspase activation are classical approaches to detect apoptosis. The degree of apoptosis can be assessed by standard techniques based on detection of DNA fragmentation such as the TUNEL method. Activities of caspases, as another marker to evaluate apoptosis, can be measured by enzyme reaction on specific substrates, protein expression with Western blotting or mRNA level through real-time PCR. Caspase response is more sensitive than TUNEL assay. Reasons for the discrepancy between the low number of TUNEL-positive cells and the high number of cells revealing caspase activation are undetermined (Borisov and Carlson, 2000; Resendes et al., 2004). They may include that (i) the inconsistency may be explained by the time course of biochemical events in apoptosis. DNA fragmentation is understood as a late event in apoptosis, whereas caspase activation occurs earlier than DNA cleavage; (ii) some forms of apoptotic hepatocytes are not always associated with DNA fragmentation (Oberhammer et al., 1993); (iii) perhaps when DNA fragmentation occurs in late stages of apoptosis, apoptotic cells are rapidly phagocytosed and escape detection by TUNEL staining. Therefore, the approach of quantifying apoptosis by morphologic criteria and DNA fragmentation results in underestimation of the actual number of apoptotic cells. Cytokeratin 18 neoantigen (M30), an early caspase cleavage event, reflects ongoing hepatocyte apoptosis (Valva et al., 2010). The M30 may be a surrogate to replace the liver biopsy for diagnosis of apoptosis.

5. Examples of liver injury

5.1. Chronic viral hepatitis

Liver is a target organ of hepatotropic viruses. Disturbance of apoptosis is implicated in infection with hepatitis C virus (HCV) that is characterized by inflammatory liver damage and a long viral persistence associated with an increased risk of developing hepatocellular carcinoma. Inflammatory cytokines/chemokines such as TNFα, TGFβ, IFN-γ, IL-10, IL-12, IL-22, CCL3, CCL4, CXCR3, IP-10, CCR5, and RANTES, are released in HCV-infected liver (Barrett