Apoptosis in selected liver diseases

Seçilmiş karaciğer hastalıklarında apoptoz

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Cell death by apoptosis usually occurs in a regulated, limited fashion. Only selected target cells are deleted in the process of ontogeny, development and regeneration. In contrast, under pathophysiological conditions, apoptosis imposes as a massive, chaotic, non-selective event that may occur for periods or even up to decades. It is likely that apoptosis is the initial cellular response to injury and may thus initiate several, intertwined cellular and cytokine cascades that culminate in tissue injury, inflammation, fibrosis and finally, in cirrhosis. Obviously, this cascade of events is of particular importance in various types of acute and chronic liver diseases. In contrast, defective apoptosis and increased cell proliferation is associated with cancer. Indeed, tumor cells often show alterations in genes regulating the apoptosis machinery. This overview is focused on the role of apoptosis in select important liver diseases. Today, rational-based strategies are being developed to either promote or suppress apoptotic cell death as a novel therapeutic option in the treatment of these liver diseases.

Key words: Apoptosis, non-alcoholic steatohepatitis, HBV, HCV, hepatocellular carcinoma, alcoholic liver disease

Apoptozla ilişkili hücre ölümü genellikle düzenli ve sınırlı şekilde oluşur. Yalnızca seçilmiş hedef hücreler oluşum, gelişim ve çoğalma sürecinde yok edilir. Bununla birlikte patofizyolojik durumlarda, apoptoz kısa bir dönem ya da bir kaç on yılı kapsayacak kadar uzun sürebilen yaygın, karmaşık ve seçici olmayan durum olarak karşımıza çıkabilir. Apoptoz muhtemelen hasara cevap olarak gelişen başlangıçtaki hücresel cevaptır ve doku hasarı, inflamasyon, fibrosis ve sirozla sonuçlanan birkaç hücresel ve sitokin kaskadını başlatabilir. Buna karşın yetersiz apoptoz ve aşırı hücre çoğalması kanserle ilişkilidir. Gerçektende, tümör hücreleri sıklıkla apoptozu regüle eden genlerde değişiklikler gösterirler. Bu derleme apoptozisin bazı önemli karaciğer hastalıklarındaki rolüne odaklanmıştır. Son zamanlarda, bahsedilen karaciğer hastalıkları için yeni tedavi seçeneği olarak apoptotik hücre ölümünü artıran ya da azaltan akla yatkın stratejiler geliştirilmektedir.

Anahtar kelimeler: Apoptoz, non-alkolik yağlı karaciğer hastalığı, HBV, HCV, HCC, alkolik karaciğer hastalığı

INTRODUCTION

Programmed cell death is a basic biological phenomenon that occurs in all organs and tissues. Between 1962-1964, it was in fact firstly discovered in the liver in the course of studies on ischemic liver injury (1). However, once identified in various other types and tissues, Kerr et al. (2) concluded that programmed cell death subserves a general homeostatic function and termed it apoptosis. Today, we know that in adults up to 1011 cells die per day by apoptosis, stunningly amassing to one's body weight within one year (3).

As in all other sites, a certain degree of hepatocyte apoptosis is characteristic of a healthy liver. Indeed, in recent years it has become obvious that development and progression of various liver diseases are associated with inordinate increase or decrease in hepatocyte apoptosis. Moreover, rather than only as a late consequence of more essential pathogenic processes, it appears that dysregulated programmed cell death itself may be a fundamental feature of most acute and chronic human liver diseases (3, 4). Still, the contribution of cell death to liver diseases is a consequence of both apoptosis and necrosis, or necroapoptosis (5).

Specifically, recent data suggest a direct link between upregulated apoptosis, the subsequent release of inflammatory mediators, and the development of fibrosis (6). Such an interrelationship has been well documented both for ethanol-induced hepatitis and non-alcoholic fatty liver disease

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(NAFLD), as well as cholestatic liver diseases, certain mutations of the α_1-antitrypsin gene, Wilson's disease, viral hepatitis, and ischemia-reperfusion injury (7). Subsequently, fibrosis initiated by sustained excessive apoptosis may eventually culminate in cirrhosis and liver failure. As an example, drug toxicity-related acute and massive hepatocellular apoptosis leads to acute liver failure with the consequence of emergency liver transplantation (8-10). In contrast, the combination of insufficient apoptosis with dysregulated proliferation may promote the development of cancer (11) (Figure 1). Dysregulated apoptosis is therefore associated with considerable morbidity and mortality in many liver diseases (3).

Apoptosis in the Pathogenesis of Liver Diseases

Apoptosis is a form of cell death characterized by organized nuclear and cellular fragmentation. It may occur via two fundamental pathways: (i) the death receptor (DR)-mediated or extrinsic pathway; or (ii) the intracellular organelle-based intrinsic pathways (12). During apoptosis, cells are fragmented into small membrane-bound so-called apoptotic bodies (AB), which are removed by phagocytosis (13-15).

Regulation of apoptotic machinery in hepatocytes is complex and is commonly triggered through activation of DRs (16, 17) (Figure 2). The intracellular pathway can be initiated by several organelles, for example, lysosomal permeabilization, pathologic alterations in the cellular storage and mobilization of calcium or in processes that are located in the endoplasmic reticulum. Intracellular DNA damage or mitochondrial dysfunction can all trigger apoptosis (18). Intramitochondrial functions often play a critical role in augmenting the apoptotic process. As a prominent example, mitochondrial release of cytochrome c is a common event in apoptosis and triggers a caspase-dependent cascade that culminates in programmed cell death (15).

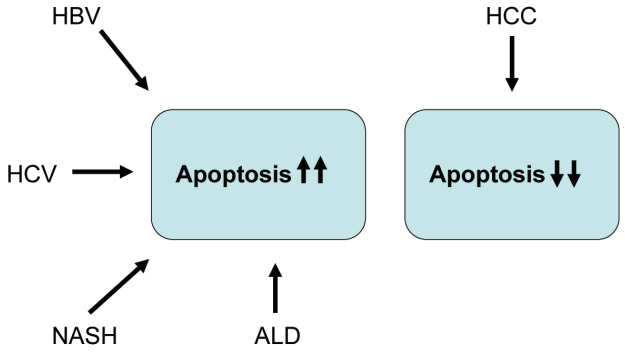

Figure 1. Simple overview of selected liver diseases associated with excessive apoptosis demonstrating the underlying pathomechanisms.

Once apoptosis was identified for its important role in fibrogenesis, the attention shifted to its potentially modulating effects on fibrogenesis (19, 20). The increased activation and proliferation of hepatic stellate cells (HSCs), combined with excessive synthesis of collagen, are known phenomena during liver fibrosis (21). Driving activated HSCs into apoptosis is one way to resolve fibrosis (22). In fact, in vitro and in vivo studies have shown that inducing apoptosis of activated HSCs is essential to the resolution of fibrosis (22-24). Indeed, activated HSCs express DRs such as Fas and DR5 (25, 26). In a study by Wright et al. (27), fibrotic rats were treated with gliotoxin to induce apoptosis in HSCs, which resulted in decreased collagen deposition. Issa et al. (28) showed that mutation in collagen 1 (col-1αIr/r) confers resistance to the action of collagenase and results in the failure to recover from liver fibrosis, the persistence of activated HSCs and diminished hepatocyte generation. Therefore, collagen 1 protects activated HSCs from apoptosis. Apoptosis, therefore, not only induces fibrosis but may also resolve fibrosis by inducing the death of HSCs. In NAFLD conditions, an upregulation of free fatty acids (FFAs) can lead to activation of HSCs. Indeed, we have recently shown in an in vitro study that FFA-activated human LX-2 stellate cells are more activated by subsequent addition of resveratrol. This red wine component not only amplified the expression of α-smooth muscle actin (SMA), a strong marker of fibrogenesis, but also the DR CD95/Fas and anti-apoptotic mediators like Bcl-2 and Mcl-1 (29). This finding raises the possibility that in obese patients with elevated FFAs, resveratrol could provoke hepatic fibrogenesis.

Liver injury not only causes hepatocyte apoptosis but also stimulates the production of pro-inflammatory chemokines. Hepatocyte apoptosis therefore appears to be in some discrete way associated with the generation of such chemokines (30-34). Indeed, Fas-mediated hepatocyte apoptosis elicits an inflammatory response in the liver that, secondarily, induces HSC activation (35, 36). Although originally thought of as a "silent process", uncoordinated and continuous apoptosis in the liver likely initiates sustained inflammation and, with ti-

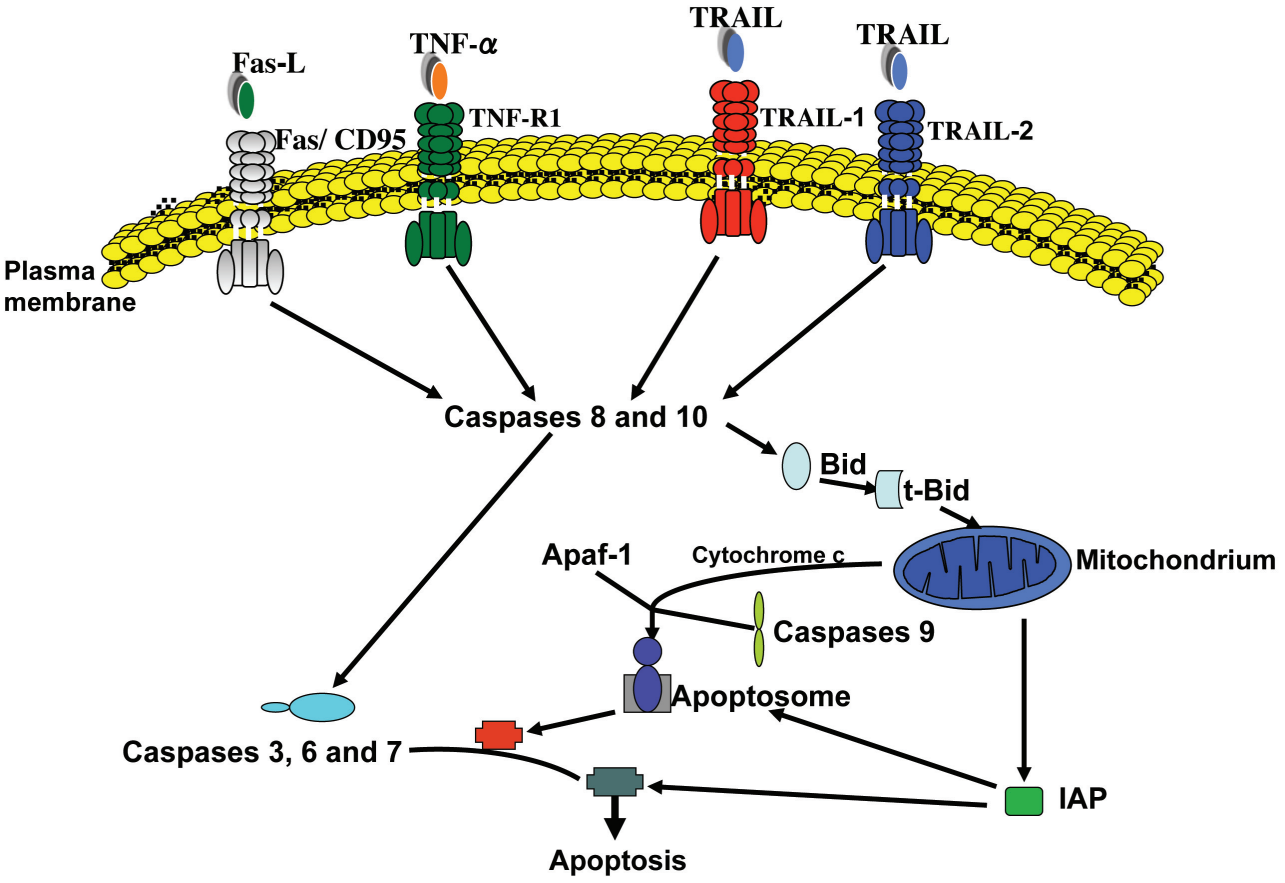

Figure 2. Death receptors (DRs) involved in hepatocyte apoptosis. Schematic representation of DR-mediated signaling in hepatocytes involving Fas/CD95, tumor necrosis factor-receptor I (TNF-RI) and the TNF-α-related apoptosis-inducing ligand receptors-1 and -2 (TRAIL-1 and -2). Receptor-ligand interaction results in receptor oligomerization and the recruitment of adaptor proteins, i. e. Fas-associated protein with death domain (FADD) and TNF-RI-associated death domain protein (TRADD). Downstream, this interaction leads to the activation of initiator caspases 8 and 10. The resulting so-called death-inducing signaling complex (DISC) can directly activate effector caspases 3, 6 and 7 and/or pro-apoptotic proteins such as Bid. These processes activate the intrinsic pathway subsequently releasing cytochrome c, and finally leading to apoptosis.

me, may act profibrogenic (6).

Liver fibrosis is a cardinal feature of chronic liver injury. Ultimately, hepatic fibrosis leads to cirrhosis with clinical complications of portal hypertension, chronic liver failure and consequently liver cancer.

Apoptosis in Certain Liver Diseases

Death receptors, particularly Fas/CD95, are broadly expressed by liver parenchyma cells and play a crucial role in the apoptotic machinery. In evolutionary terms, this may result from adaptive processes that allow the more efficient elimination of hepatocytes that have been infected, for example, with hepatotropic viruses. However, the same efficient clearance system may erroneously aggravate pathologic processes in the liver, thus demanding the conception of suitable therapeutic options.

A - Apoptosis in non-viral liver diseases

Apoptosis in non-alcoholic fatty liver disease (NAFLD)

NAFLD is the most common liver disease in developed countries (37). Liver injuries in NAFLD comprise a spectrum of steatosis, steatohepatitis, advanced fibrosis, and cirrhosis (38). The spectrum of symptoms in an advanced sub-entity of NAFLD, termed non-alcoholic steatohepatitis (NASH), comprises steatosis, balloon degeneration, inflammation and various degrees of fibrosis (39). NASH is diagnosed if the NASH scoring system (NAS) is ≥5 (40).

It is generally believed that simple steatosis is a benign condition that only holds a minimal risk of progression, whereas NASH may progress to cirrhosis (41). The best known risk (co-factor) for

NAFLD is the metabolic syndrome (including obesity, hyperlipidemia, and diabetes mellitus) and other metabolic diseases (42-44). However, although these conditions may be pivotal for developing NAFLD, the concrete pathway to NASH remains elusive. Nevertheless, more recent studies suggest that the progression in both NAFLD and NASH (where Fas and tumor necrosis factor receptor type-I [TNF-RI] have also been found to be overexpressed) correlates with increased hepatocyte apoptosis, thus possibly indicating its etiopathogenic role (45, 46). Moreover, recent findings indicate a strong interaction between adipose tissue and liver cells. Therefore, it is likely that the progression to NASH in NAFLD patients is hormonal, regulated by the adipocytes (47).

It has been shown that in high carbohydrate-fed mice (thus evaluating one key risk factor for developing NAFLD), the Fas receptor is significantly upregulated. Fas-induced hepatocyte apoptosis is mediated by the caspase 8-dependent cleavage of Bid, a pro-apoptotic member of the Bcl-2 family that translocates to mitochondria and, in concert with other pro-apoptotic proteins (e.g. Puma, Noxa, Bim), induces the loss of mitochondrial integrity (48). Successive release of cytochrome c then activates the apoptosis machinery via caspases-3 and -7 (45, 49). Additionally, the well-documented release of reactive oxygen species (ROS) upon mitochondrial disruption further exacerbates liver injury and inflammation (11, 50). The determination of M30, a neo-epitope that detects cytokeratin 18 fragments, strongly predicts apoptosis-dependent processes in patient with NASH (51).

Apoptosis in ethanol-induced liver disease

The pathogenesis of ethanol-induced liver injury is still unknown. However, recent experimental data again support a crucial role of hepatocyte apoptosis (50). In mice, chronic ethanol instillation results in a significant time-dependent increase in hepatocyte apoptosis. Similar to the situation in humans, these changes are potentially reversible after ethanol withdrawal (52). Interestingly, while hepatocyte Fas levels in alcohol addicts were as low as in non-alcoholics, their FasL mRNA levels were found to be significantly increased. In these patients, hepatocyte death may thus be induced via paracrine or autocrine pathways (53).

Hepatocyte apoptosis directly correlates with disease severity, bilirubin and alanine aspartate (AST) levels and the degree of steatosis (54, 55). Such hepatocytes often co-localize with infiltrating neutrophils, which suggests a subsequent inflammatory response to liver injury. Several mechanisms have been proposed to explain induced hepatocyte apoptosis. One prominent target is the cytochrome P450 isoform, CYP2E1. The metabolization of ethanol, especially via CYP2E1, leads to release of ROS and the generation of lipid peroxidation products (11). ROS may cause mitochondrial dysfunction and affect the release of pro-apoptotic factors, such as cytochrome c, with the consequence of caspase activation (11, 50). In line with this, antioxidants have been shown to reduce apoptosis in ethanol-exposed rats (56, 57). ROS may increase FasL mRNA expression in hepatocytes and activate autocrine or paracrine mechanisms of cell death.

Another possible mechanism is the TNF-α pathway, involving nuclear factor kappa B (NFκB) activation, which can upregulate the expression of Fas and FasL genes (58). Indeed, increased TNF-α serum levels that contribute to liver injury have been found in patients with ethanol-induced hepatitis (59). Moreover, the expression of the high- and low-affinity TNF-α receptors (TNFα-RI and -RII) is much increased in chronic ethanol abuse, thus sensitizing for TNF-α-induced apoptosis. Indeed, some studies have shown an improvement in alcohol-induced hepatitis after anti-TNF-α treatment (60, 61). However, in October 2002, a multicenter randomized trial with the anti-TNF-α antibody infliximab in patients with severe alcoholic hepatitis was stopped by the French drug agency. The study aimed to determine the superiority of infusions with infliximab (10 mg/kg) associated with prednisolone (40 mg/day) compared with infusions of placebo. The main end-point was the two-month mortality rate. An analysis was performed after the inclusion of 36 patients. Unexpectedly, there was a two-fold increase in death in the infliximab versus the placebo group. The main causes of death were due to infection, hemorrhage and very often renal failure (62). However, because of the risk of severe adverse events - already described in patients with Crohn's disease - the results of the French multi-center trial should be fully discussed before starting a new trial, including the choice of the infliximab dose.

B - Apoptosis in viral liver disease

The hepatitis B and C viruses (HBV, HCV) are two main causative agents for virus-induced liver damage and cirrhosis. Persistent infection of he-

patocytes for years may in general lead to liver inflammation and, in the course of this process, hepatocytes undergo a high rate of cell destruction and regeneration that results in an increased risk of developing hepatocellular carcinoma (HCC) (11). Virus-induced liver injury is mainly a consequence of the host's immune response to viral proteins expressed by infected hepatocytes or of the direct cytopathic effects of these viruses.

Specifically, T-lymphocytes recognize and kill hepatocytes expressing viral antigens to clear the virus from the liver, which is the first cause of liver damage (63). In addition, signals released from these immune cells promote liver inflammation and chemotactically attract further inflammatory cells such as neutrophils (64). Several studies have shown that the elimination of virus-infected hepatocytes by cytotoxic T-cells mainly occurs via DRs, with Fas as the most prominent example (65, 66). Increased Fas and FasL expression has actually been detected in virus-infected patients and correlates with severity and location of liver inflammation. Fas expression can be induced by both the expression of viral protein or inflammatory cytokines (65, 66). Perhaps the two most important cytokines to this effect are interleukin-1 (IL-1), as generated early in the anti-viral response, and TNF-α, which acts as a pleiotropic late stage mediator (63, 67).

Hepatitis B

It was demonstrated that the T cell-mediated perforin death pathway as well as the Fas/FasL system play critical roles in liver cell damage in chronic HBV infection (68). In any event, it has been shown that selected HBV proteins interfere with mitochondrial pathways (69); it may then indeed depend upon flanking circumstances whether such interaction rather stimulates or suppresses a cell's apoptotic machinery.

Another example is the x protein of HBV (HBx). As a potent transactivator, it is essential for viral replication. In studies on transgenic mice, HBx stimulated apoptosis of hepatocytes by sensitizing them to death ligands such as TNF-α and TNF-α-related apoptosis-inducing ligand receptors (TRAIL) (70). In contrast, HBx can also stimulate the NFκB and JNK pathways that are known to block Fas-mediated hepatocyte apoptosis (71).

HBxAg also appears to promote fibrogenesis, by stimulating the production of fibronectin. HBxAg also stimulates the production and activity of transforming growth factor (TGF)-β1 by several mechanisms, thereby promoting the pro-fibrogenic and tumorigenic properties of this important cytokine. In addition, HBxAg appears to remodel the extracellular matrix (ECM) by altering the expression of several matrix metalloproteinases (MMPs), which may promote tumor metastasis. Hence, HBxAg appears to promote chronic infection by preventing immune-mediated apoptosis of infected hepatocytes, by promoting the establishment and persistence of fibrosis and cirrhosis preceding the development of HCC, and by promoting the remodeling of EMC during tumor progression (72). These findings suggest the relative balance between these processes to actually determine whether pro- or anti-apoptotic stimuli may eventually prevail. As a result, increased apoptosis may support liver injury, while its inhibition may maintain chronic infection and thus, indirectly promote carcinogenesis. The rate of hepatocellular apoptosis in acute liver failure induced by HBV infection is higher than in acetaminophen-induced liver failure (unpublished data).

Hepatitis C

Furthermore, it has recently been published that caspase activation is considerably increased in HCV-infected livers and correlates with the inflammatory response and fibrosis-related processes (73, 74). In line with these findings, HCV patients reveal elevated serum levels of caspase-generated cleavage fragments of cytokeratin 18 (M30) (73, 75). Therefore, besides supporting the understanding that apoptosis promotes fibrosis, these authors identified a new, more sensitive biomarker for detecting apoptosis-related liver injury. It may now be employed diagnostically as well as a therapeutic read-out marker indicating the efficacy of (co-)treatments with interferon-α or other therapeutic approaches (76).

As yet, it could not be clarified how selected viral proteins influence the apoptotic process in the diseased liver. In fact, HCV proteins either support or inhibit the apoptosis of hepatocytes, and it appears that the promotion of either of these alternatives depends on the specific circumstances and/or the classes of viral proteins involved. For example, in a human hepatoma cell line, the HCV core protein increases susceptibility to DR-mediated apoptosis (77), while the core, E1, E2 and NS2 proteins, when expressed in transgenic mice, inhibit the same process (78, 79). In addition, HCV can manipulate the immune system of the host, disrupting

both innate and adaptive immunity to establish persistent infection. The immune system initially attempts to eradicate the virus, but in the setting of chronic infection, probably promotes hepatocyte damage and fibrosis through direct cellular toxicity and the release of inflammatory cytokines. Multiple types of cytotoxic lymphocytes, comprising the unique immune hepatic microenvironment, are likely to be important in the pathogenesis of HCV-induced liver damage. The net liver damage from HCV infection depends on the balance between the host's anti-viral mechanisms and the virus's ability to subvert them (80).

C - Apoptosis in hepatocellular carcinoma (HCC)

In 90-95% of all primary liver tumors, HCC is the most common malignancy arising from parenchymal cells (81). Various factors associated with its pathogenesis include chronic viral hepatitis, NASH, ethanol consumption, hereditary diseases (α_1 antitrypsin deficiency, hemochromatosis), and exposure to hepatotoxins (aflatoxin) (82, 83).

In its progressive stage, HCC has been particularly associated with both defective apoptosis and increased cell proliferation. Tumor cells often show alterations in genes regulating the apoptotic machinery (84). Indirectly, these also include mutations of the tumor suppressor gene p53 (85, 86). Expressed in response to DNA damage, p53 may induce a cell-cycle arrest allowing DNA repair. However, in the face of extensive damage, p53 engages pro-apoptotic BH3-only proteins and Bcl-2 homologues (Puma, Noxa, Bid and Bax) to eliminate the affected cell by apoptosis. p53 can further upregulate DRs (Fas, TRAIL-R1) and their ligands (FasL, TRAIL) (87). Dysfunctional p53 thus allows damaged cells to escape their apoptotic clearance and progress to cancerous proliferation (88). Similar processes may result from the loss of another putative tumor suppressor, TIP 30/CC3 (89), or from HCV infection (90). In both cases, aberrant cells are no longer efficiently eliminated. This leads to the development of different types of cancer that include HCC (89) and cancer of the hepatobiliary tract, or hilar cholangiocarcinoma, respectively (90).

By causing DNA damage, chemotherapeutics often aim at inducing tumor cell apoptosis via p53 activation. However, resistance to such treatment by p53-impaired tumors entails a bad prognosis. Therefore, several clinical studies currently probe a beneficial effect of exogenous p53. As for HCC, the introduction of wild-type p53 by adenoviral vectors already bears some promise for an improved therapeutic outcome (91, 92).

Besides the known p53 effects on the apoptosis machinery, alteration in the expression of DRs also leads to defective apoptosis. Indeed, in HCC, Fas is downregulated while its ligand is highly expressed, thereby allowing cancer cells to protect themselves and to kill immune cells alike. Several studies have shown that reduced Fas expression in HCC negatively correlates with patient survival (93, 94). It has also been found that the anti-apoptotic protein Bcl-XL is overexpressed in HCC, which confers resistance to mitochondria-mediated apoptosis. In HCC, Bcl-XL, therefore, also contributes to Fas resistance. Since Fas-mediated apoptosis in hepatocytes is linked to a mitochondrial pathway, it may be envisioned that certain components of this pathway can therapeutically be targeted by novel HCC-directed drugs (11).

Meanwhile, promising approaches have been made towards treating hepato-malignancies with new potent inducers of apoptosis. First, Jing et al. (94) found that emodin induces apoptotic responses in the HCC lines Mahlavu, PLC/PRF/5 and HepG2 (hepatoma cell line), thus effecting a time- and dose-dependent growth inhibition. Specifically, ROS generated in response to emodin treatment caused a reduction in the mitochondrial transmembrane potential ($\Delta\Psi_M$) that was followed by the activation of caspases 9 and 3 and led to DNA fragmentation and apoptosis. Second, in HepG2 cells, application of the cyclooxygenase 2 inhibitor, NS398, led both to a concentration-dependent inhibition of cell proliferation as well as to the induction of apoptosis. Mechanisms likely responsible for these beneficial processes were a reduction of cells being in the S-phase and an accumulation of quiescent G0/G1 cells as well as a decrease in Bcl-2 expression (95). Finally, in human HCC-9204 cells transfected with complete Bax cDNA, induced overexpression of Bax led to the induction of apoptosis and moreover, sensitized HCC cells to adriamycin-induced apoptosis (96).

Recently, it has been shown that resveratrol, a red wine integrant, acts as a potent sensitizer for anti-cancer drug-induced apoptosis by inducing cell cycle arrest (97, 98). Indeed, application of resveratrol resulted in cell cycle arrest in the S phase and apoptosis induction preferentially out of the S phase upon subsequent drug treatment. Resverat-

rol-mediated cell cycle arrest sensitized for apoptosis by downregulating survivin (IAP, inhibitor of apoptosis proteins) expression through transcriptional and post-transcriptional mechanisms (99).

Finally, recent evidence indicates that inflammatory processes, as well as the epithelial-mesenchymal transitions that occur in HCC cells to facilitate their dissemination, are related to cell survival (100). Therefore, therapeutic strategies to selectively inhibit anti-apoptotic signals in liver tumor cells have the potential to provide powerful tools to treat HCC. Similar to the promising findings potentially enabling improved therapeutic onsets for those liver diseases that coincide with excess apoptosis, these and other ongoing studies also let us anticipate much more promising treatments for liver malignancies that perhaps may even address further progressed stages of disease.

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