Modulation of programmed cell death pathways by the hepatitis C virus

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2. CONTRIBUTION OF STRUCTURAL PROTEINS TO APOPTOSIS REGULATION

2.1. Core

The core protein is encoded by the N-terminal portion of the HCV precursor polyprotein and cleaved from the polyprotein by cellular signal peptidase to give the immature form of the core protein (residues 1 to 191). This then is further cleaved by membrane-associated signal peptide peptidase to give the mature core protein, whose C terminus is not precisely known but lies between residues 170 and 179 (see reviews (13-15)). Besides its role in the encapsidation of viral RNA, core has been found to interfere with many cellular pathways, including cell signaling, transcriptional activation, lipid metabolism, carcinogenesis, and apoptosis (see reviews (13-15)).

Depending on the death stimuli and types of cells used, core has been reported to inhibit as well as promote apoptosis. As both the immature and mature forms of core have been used in these studies, some differences in the activity of core observed may also be related to the form of core expressed. A summary of the different apoptotic pathways that are affected by core is provided below. Many of these studies were performed by ectopic expression of core alone and should be re-examined in the context of productive HCV replication where the other HCV proteins are also expressed. In particular, the HCVcc system can be used to determine if the level of core in the infected cells is sufficient to modulate the specific apoptotic pathways. Overall, it appears that multiple domains present in the core protein contribute to the modulation of apoptosis via diverse pathways. Therefore, the net apoptotic effect of the core protein may be dependent on the relative strength of its prosurvival and proapoptotic properties. However, the relative contribution of these various factors to apoptosis induction during HCV infection remains to be determined.

2.1.1. Effects of core on extrinsic apoptotic pathways
Core has been shown to interact directly with various components of the extrinsic apoptotic pathways, resulting in sensitization to Fas- and TNF-mediated apoptosis. Core was found to act as a positive regulator of Fas-mediated apoptosis in several cell lines (16-18). Interestingly, the C-terminal domain (residues 153 to 192) facilitates Fas oligomerization and is required for apoptosis induction in Jurkat cells in a Fas ligand-independent manner (18). Core has also been shown to bind the death domain (DD) of TNF receptor-1 as well as one of its signaling molecules Fas-associated death domain protein (FADD) (19, 20). Consequently, the core-expressing cells are sensitized to TNF-mediated apoptosis. As for TRAIL (also called Apo2L), which is a novel TNF superfamily member with strong homology to Fas ligand, there is one study that showed that core enhances TRAIL-mediated apoptosis via a mitochondrial-dependent signaling pathway (21).

On the other hand, core seems to inhibit Fas- or TNF-mediated apoptosis via several indirect pathways. These include the activation of nuclear factor kB (NF-kB) and the upregulation of inhibitor of caspase-activated DNase (22, 23). Core has also been shown to inhibit caspase-8 activation by sustaining the expression of cellular FLICE (FADD-like interleukin-1β-converting enzyme)-like inhibitory protein (c-FLIP) (24).

2.1.2. Effects of core on intrinsic apoptotic pathways

Core is localized to various cellular compartments including the surface of lipid droplets, nucleus, endoplasmic reticulum (ER) and mitochondria (see recent review (25)). As mitochondria are key sites for the regulation of apoptosis induced by intrinsic stimuli, it is not surprising that core has a direct functional effect on mitochondria. In transgenic mice and various cell lines, the expression of core causes an increase in mitochondrial reactive oxygen species (ROS) production and lead to apoptosis induction (26-32). The precise mode of action that leads to increased ROS production is not clearly defined but the N-terminal domain (residues 1 to 75) of core has been shown to interact with Hsp60, a stress response molecular chaperon that is primarily compartmentalized in mitochondria matrix (32). The interaction between core and Hsp60 was found to be important for the increased ROS production and the sensitization of the cells to apoptosis while the overexpression of Hsp60 rescued the core-expressing cells from apoptosis (32).

It was also reported that core increases the mitochondrial Ca²⁺ uptake and causes oxidation of the glutathione pool (26). Subsequently, there is a reduction in the activity of the electron transport complex I and an increase in ROS production (26). Another study showed that core stimulates the mitochondrial Ca²⁺ uniporter activity, resulting in increased mitochondrial Ca²⁺ uptake in response to ER Ca²⁺ release (27). Interestingly, core also triggers apoptosis by inducing ER stress and causing ER Ca²⁺ depletion (33). Subsequently, mitochondrial dysfunction occurs due to the changes in Ca²⁺ homeostasis. Thus, core-induced apoptosis may be due to a combination of actions at both the ER and mitochondria.

The family of Bel-2 proteins plays important roles in regulating the mitochondrial apoptotic pathway (34, 35). There are at least 12 different members in the Bcl-2 family and they may be classified broadly into three classes: prosurvival members containing multiple Bcl-2 homology (BH) domains, proapoptotic members containing multiple BH domains, and proapoptotic members containing the BH3 domain only. Members of the Bcl-2 family are also involved in viral infections as numerous viruses have been shown to encode homologs of prosurvival Bcl-2 proteins, and these viral proteins act to inhibit apoptosis in infected cells and prevent the premature death of these cells (see reviews (36, 37)). Recently, a Bcl-2 homology domain 3 (BH3) in the core protein of the HCV-S1 strain (genotype 1b) was identified and shown to be essential for its proapoptotic property (38). Core appears to be a bona fide BH3-only protein having properties similar to those of Noxa, a BH3-only member of the Bcl-2 family that binds preferentially to Mcl-1, a prosurvival member of the Bcl-2 family. Interestingly, the genotype 1b core protein is more effective than the genotype 2a core protein (of the JFH-1 strain) in inducing apoptosis due to a single-amino-acid difference.

However, two independent studies reported that core can exert prosurvival effects at the mitochondria (29, 39). Using transiently transfected and genetically collected core-producing HepG2 and Huh7 with core expression under control of the Tet-Off promoter, these studies reported that the mRNA and protein levels of the pro-survival Bcl-XL were increased by core. Subsequently, these cells are more resistant to apoptosis induced by deoxycholic acid, TNF-alpha (with actinomycin D) or Fas stimulation.

2.1.3. Effects of core on other cellular pathways that contribute to apoptosis regulation

The effects of core on several cellular pathways also lead to the induction of apoptosis indirectly. For example, core interacts with 14-3-3ε, resulting in the release of Bax from the Bax/14-3-3ε complex in the cytoplasm and activation of apoptosis (40). One important consideration may be the cellular localization of different forms of core as this will impact on core’s interaction with host-factors. Indeed, it was reported that the effect of core on p53 and p21 activities is dependent on its subcellular location (41, 42). As such, core has been shown to promote as well as inhibit p53-mediated apoptosis (41, 43, 44). The proapoptotic effect of core was also reported to be enhanced by nuclear localization and found to be correlated to protein kinase R (PKR) activation (45).

2.1.4. Effects of alternate reading frame proteins on apoptosis

HCV has an alternate reading frame (ARF) that overlaps the core protein gene and several ARF proteins are expressed (see recent reviews (46, 47)). There are limited studies on these proteins but one study reported that one of these ARF proteins, termed as the F protein, can inhibit TNF-mediated apoptosis by activating the NF-κB pathway (48).

2.2. E1 and E2

HCV encodes two glycoproteins, E1 and E2, which interact to form complexes on the surface of the virion and mediate viral attachment and entry through interaction with cellular receptors (see recent review (49)). Beside this role, E1 and E2 are
also involved in modulating cellular functions when they are expressed intracellularly. For example, the overexpression of E1 induced apoptosis in HepG2 cells and this could be partially blocked by caspase inhibitors (50). The induction of apoptosis was also found to be dependent on the transmembrane domain at the C terminal of E1 and may be due to the alternation of ER membranes by E1.

The effects of E2 on apoptosis regulation are more complicated as both proapoptotic and prosurvival effects have been observed. When E2 was transiently expressed in various cell-lines, including HuH7, apoptosis was observed (51, 52). Expression of E2 caused cytochrome c release and cleavage of Bid, suggesting that a mitochondrial pathway is involved (52). However, the binding of recombinant E2 to the surface of HuH7.5 or Daudi cells was also sufficient to cause a decrease in cell viability (53, 54). This suggests that E2 can also induce apoptosis presumably via an “innocent bystander” mechanism that resulted from the binding of E2 to CD81 on the cell surfaces. In contrast, the binding of E2 to primary thyroid cells did not induce apoptosis but caused secretion of IL8 (54).

Interestingly, Munshi and co-workers showed that E2 can act cooperatively with the envelope protein of the human immunodeficiency virus type 1 (HIV-1), gp120, to induce apoptosis in HepG2 cells (55). Similar to the studies described above, this involves an “innocent bystander” mechanism that resulted from the cell surface binding of these proteins and is independent of viral replication (55). Further studies revealed that the apoptosis induction is caused by activation of the signal transducer and activator of transcription factor 1 (STAT1) which leads to increased Fas ligand expression and translocation of Bid to the mitochondria (56, 57). As the potential cross-talk between HIV-1 and HCV may affect the treatment of HIV-1 and HCV co-infected patients (see recent review (58)), it is important to examine the clinical significance of these findings in future studies.

However, prosurvival activity was observed in stable cell lines expressing E2 (59). Replicon-containing HuH7 cells that stably express core, E1 and E2 showed resistance to TRAIL-induced apoptosis when compared to the parental HuH7. As this effect was not observed in replicon-containing HuH7 cells that stably express core and E1, it seems that E2 is responsible for the prosurvival effect and this is confirmed by experiments performed using HuH7 cells stably expressing E2 only. Furthermore, the level of several survival genes related to NF-κB activation were found to be up-regulated in the E2-expressing cells (60). One of them is the molecular chaperone glucose-regulated protein (GRP) 94 and RNA interference experiments showed that GRP94 is essential for the resistant to TRAIL-induced apoptosis in both E2-expressing cells or HuH-7 infected with JFH-1 recombinant HCVcc (60). Taken together, the HCV E2 modulates apoptosis via several pathways. The difference in the property of E2 observed in the various studies may be due its ability to modulate extrinsic and intrinsic apoptotic pathways differently.

3. CONTRIBUTION OF NON-STRUCTURAL PROTEINS TO APOPTOSIS REGULATION

Besides the structural proteins, HCV also encodes 6 non-structural proteins and a small p7 protein, which has not been formally designated as structural or non-structural. For simplicity, p7 is classified as non-structural in this review. With the exception of NS4B, all of these proteins have been shown to have either proapoptotic or prosurvival or both activities (Figure 1). This is summarized in this section.

3.1. p7

The p7 protein is a small highly hydrophobic protein located at the junction between the structural and nonstructural proteins (5). P7 has ion channel activity and is thus classified in the family of viral ion channel proteins known as viroporins (61, 62). Recently, Madan et al demonstrated that HCV p7 protein induces caspase-dependent apoptosis in baby hamster kidney cells (63). However, the level of apoptosis induced by p7 seems to be much lower than that induced by other viroporins (63).

3.2. NS2

NS2, one of the two viral proteases required for the post-translational cleavage of the nonstructural proteins, is a hydrophobic transmembrane protein anchored to the ER (5). Erdtmann et al demonstrated that NS2 was able to interact with and inhibit apoptosis induced by the liver-specific pro-apoptotic CIDE-B protein. The prosurvival activity of NS2 is believed to be related to its ability to interfere with the cell-death pathway by binding to the CIDE-B cell death domain (64).

3.3. NS3

HCV NS3 is a multifunctional protein believed to be involved in several essential processes in the virus lifecycle, such as protease, nucleotide triphosphatase and RNA helicase activity (5). Apart from these enzymatic activities, NS3 has been reported to have both proapoptotic and prosurvival activities. For instance, in actinomycin D treated cells, NS3 was shown to suppress apoptosis by forming a complex with the tumor suppressor p53, and thus decreasing the amount of p53 through a decrease in transcription and/or translation of p53 or through the degradation of p53 (65, 66).

On the other hand, Stavoshian et al, demonstrated that NS3 was proapoptotic in mature dendritic cells and that the proapoptotic effect was due to its ability to down-regulate the expression of p21WAF1/CIP1 (67). Similarly, when expressed in human and simian cells by transient transfections, NS3 and its precursor NS2/3 have been shown to induce caspase-8-mediated apoptosis (68). Site-directed mutagenesis showed that the proapoptotic activity of NS3 is independent of its protease and helicase activities (68). In phagocytes, the expression of NS3 led to the release of ROS from phagocytes by activating the enzyme, reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (69). The activated phagocytes subsequently induced apoptosis in both T cells and NK cells (69).
The discrepancies in reports of the apoptotic ability of NS3 may be partly due to the different cell lines used in the studies. For instance, it was observed that the induction of apoptosis is higher in Vero and HepG2 cells than in Huh-7 cells (68).

3.4. NS4A

HCV NS4A is a small protein which acts as a cofactor for NS3 in its protease activity (5). NS4A is reported to be proapoptotic because it has a mitochondrial-damaging effect (70). For instance, using Huh-7 cells, it has been shown that NS4A is able to decrease the transmembrane potential of the mitochondrial resulting in the release of cytochrome C and the activation of caspase-3 followed by cell death (70). Just like p7, NS4A has been shown to have ion channel activities and is classified as a viroporin (63). Among the various viroporins tested for proapoptotic activities, NS4A caused the most rapid induction of caspase-3 activation and triggered the release of cytochrome C efficiently (63).

In two separate studies, NS3 and NS4A were expressed as a complex but opposite effects on apoptosis were observed. The NS3/4A complex was reported to inhibit apoptosis induced by the mitochondrial antiviral signaling adaptor protein (MAVS), also known as IPS-1, VISA or Cardif, by cleaving MAVS at its transmembrane domain, resulting in its dissociation from the mitochondria and thus destroying its proapoptotic functions (71). In contrast, Nomura-Takigawa et al. observed that the NS3/4A complex sensitized cells to actinomycin D-induced mitochondria-mediated apoptosis (70).

3.5. NS5A

The HCV NS5A protein is a phosphoprotein which interacts with several host proteins and signaling pathways (72, 73). With regards to apoptosis, it has been shown to inhibit both extrinsic and intrinsic apoptotic stimuli. For example, NS5A has been shown to inhibit TNF-α mediated apoptosis (74-76). The only exception was observed in mature dendritic cells where the overexpression of NS5A induced apoptosis (67).

The prosurvival activities of NS5A appear to result from its ability to interact with various host proteins. For example, NS5A is a potential viral Bcl-2 homologue and is able to bind Bax via its BH2 domain, thus inhibiting apoptosis by sequestering Bax in the nucleus (77). The BH1 domain in NS5A was also shown to interact with the Fas506-binding protein 38 (FKBP38) and the prosurvival activity of NS5A was reduced when the expression of endogenous FKBP38 was suppressed by RNA interference (78). On the other hand, a Src homology 3 domain in NS5A was found to be responsible for its interaction with Bin-1 and inhibition of Bin-induced apoptosis (79). Other studies linked the prosurvival activity of NS5A to its ability to bind p53, PKR protein kinase, Grb 2 or p85 phosphatidylinositol 3-kinase (80-83). It also has the ability to activate a calpain cysteine protease, leading to the degradation of Bid, and inhibit the oxidative-stress induced p38 MAPK phosphorylation of Kv2.1 (84, 85). Both of these resulted in dampening of the apoptotic signaling.

3.6. NS5B

HCV NS5B is the viral RNA-dependent RNA polymerase (5). Thus far, it has only been shown to induce apoptosis in mature dendritic cells (67).

4. APOPTOSIS DURING INFECTION BY RECOMBINANT HCVcc

With the production of recombinant HCVcc, it becomes clear that HCV infection of hepatoma cells leads to cell death with numerous hallmarks of apoptosis like exposure of phosphatidylserine on the outer surface of the plasma membrane, activation of caspase 3, nuclear condensation and DNA fragmentation (86-89). The mitochondrial pathway was clearly affected by HCV infection as it triggers Bax activation, leads to disruption of mitochondrial transmembrane potential and release of cytochrome C (89). In addition, there is oxidative stress due to the enhanced level of mitochondrial ROS (89-92). This could be related to the ability of core to increase mitochondrial ROS (see above).

However, it is unclear if ER stress is also involved in HCVcc-induced apoptosis. ER stress markers, like GRP78, was found to be upregulated in one study (88) but another (89). The difference may be due to the use of different strain of virus (JFH-1 or intragenotypic chimeric J6/JFH-1) or multiplicity of infection. However, HCV infection has also been shown to induce ER stress and sensitize infected cells to apoptosis in the SCID/Alb-uPA mice (93). Studies using the subgenomic replicon systems also suggested that HCV replication disrupts normal ER functions and induces ER stress (see review (94)). Thus, it appears that ER stress can contribute to HCV-induced apoptosis but the induction of mitochondrial ROS and HCV-induced apoptosis can also occur independently of ER stress.

Recently, a Bcl-2 homology domain 3 (BH3) in the core protein of the HCV-S1 strain (genotype 1b) was identified and found to be essential for its proapoptotic property (38). Interestingly, the genotype 1b core protein is more effective than the genotype 2a core protein (of the JFH-1 strain) in inducing apoptosis due to a single-amino-acid difference. Consistently, substitution of this residue in the J6/JFH-1 infectious clone (genotype 2a) with the corresponding amino acid in the genotype 1b core protein produced a mutant virus, J6/JFH-1(V119L), which induced significantly higher levels of apoptosis in the infected cells than the parental J6/JFH-1 virus. Interestingly, more progeny virus is released from cells infected with the J6/JFH-1(V119L) virus than by those infected with the parental J6/JFH-1, while there is no difference in the efficiency of infection or amount of HCV replication inside the cells. The enhanced releases of virus from infected cells that are undergoing apoptosis have been reported for other viruses, like the infectious bursal disease virus, adenovirus, and Aleutian mink disease parvovirus (95-97), indicating that apoptosis can be advantageous for viral spreading at the late stages of infection. Mateu and co-workers also
reported that a correlation between the level of infectious particles produced and the level of cell-death in Huh7 transfected with intragenotypic JFH1 based viral RNA (87).

In another study, it was found that HCVcc infection sensitizes Huh7.5 cells and primary human hepatocytes (PHH) to TRAIL-induced apoptosis (98). Furthermore, this enhancement was shown to be mediated by the HCV nonstructural proteins and is caspase-9 dependent. A low level of apoptosis was also observed in the HCV RNA transfected Huh7.5 cells but the level of apoptosis increased dramatically upon TRAIL treatment. This indicates that there is a synergistic activation of both the extrinsic and intrinsic apoptotic pathways. In another hepatoma cell-line, LH86, massive apoptosis was also observed upon HCV infection (86). Interestingly, HCV infection of LH86 cells caused a significantly higher level of increase in TRAIL mRNA levels when compared to infected Huh7.5 cells and this seems to be correlated to the higher level of apoptosis observed in LH86 cells (86). Furthermore, it was demonstrated that IRF-3 is responsible for both IFN beta production and TRAIL induction in infected LH86 cells (86).

Most of the current HCVcc studies are based on the genotype 2a JFH-1 genome or intragenotypic JFH-based genomes. On the other hand, most of the ectopic expression studies (described above) were performed using viral proteins from genotype 1a or 1b HCV strains. It is expected that future studies will reveal more about apoptosis regulation during infection especially when infectious clones of other genotypes become available.

5. INVOLVEMENT OF OTHER CELL-DEATH PATHWAYS

Apoptosis is the most well-characterized cell death pathway during viral infection. However, emerging evidence suggests that non-apoptotic cell death pathways are also highly regulated cellular suicide processes and contribute to pathological injury (99-101).

Autophagy is a highly regulated cellular process, by which cytoplasmic materials are enclosed within double (or multiple) membrane vesicles and shuttled to lysosomes for their degradation, and the participation of host autophagic machinery in viral infection is increasingly being recognized (102, 103). Several independent studies have shown that HCV replication induces an autophagic response in hepatocytes (104-108). RNA interference experiments revealed that the autophagy machinery is important for HCV replication (104, 106, 107). However, the autophagy machinery may affect more than one step in the virus life cycle. Dreux and co-workers showed that autophagy proteins are need to initiate HCV RNA translation/replication of the incoming viral RNA in the HCVcc system but they are not essential in maintaining it once these processes are established in the replicon system (107). On the other hand, the study by Tanida and co-workers suggests that autophagy proteins are involved in the production of infectious HCV particles (106).

Another major form of cell-death is necrosis which was initially thought to be an uncontrolled form of cell death, but recent studies have revealed that programmed cell necrosis, also known as necroptosis, is a genetically controlled pathway (99, 100,109). There is a lack of systematic studies on the role of necroptosis in HCV infection. A recent study showed that the core protein can induce caspase-independent cell death (110). However, typical apoptosis-associated morphological features were observed and it is not yet determine if autophagic cell-death or necroptosis is involved. Unlike apoptosis, the contributions of the different HCV proteins to other forms of cell-death have not been extensively studied and it is likely that future studies will reveal more about their roles in HCV replication and pathogenesis.

6. FUTURE STUDIES

Extensive studies have been performed to understand the mechanisms by which HCV proteins regulate apoptosis. In addition, recent studies have characterized the cell death pathways that are activated in HCV infected cells by using the genotype 2a JFH-1 (or intragenotypic chimeric of JFH-1) HCVcc system. Further studies are need to delineate the contributions of the different HCV proteins to the regulation of apoptosis, as well as other cell death pathways, and understand the role of viral apoptosis at different stages of the HCV life-cycle. Advances in the development of a small animal HCV infection model will be necessary to provide additional insights into the contributions of cell death to HCV replication and pathogenesis. Potential genotype differences will also be addressed when infectious clones of different genotypes are successfully generated. Research on the interaction between HCV and cell death pathways will eventually lead to a better understanding of intricate interplay between HCV and its human host and may contribute to the development of antiviral therapeutics and design of efficient strategies for disease control.

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8. REFERENCES


Hepatitis C virus and programmed cell death

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Figure legends
Figure 1. Schematic diagram showing the HCV proteins and their involvement in apoptosis regulation. Upward arrows indicate proapoptotic activities while downward arrows indicate prosurvival activities. Alternate reading frame proteins are not indicated. UTR: untranslated region, IRES: internal ribosome entry site.

Running title
Hepatitis C virus and programmed cell death