Apoptosis in chronic hepatitis C

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APOPTOSIS

Apoptosis - type I programmed cell death
It is a highly regulated and controlled process during cell lifecycle that may occur in *Eucaryota* organisms.
• plays a role in embryonic development and morphogenesis,
• counteracts proliferation
• controls process of the elimination of defective/damaged/infected cells
• crucial for the maintenance of tissue homeostasis
**APOPTOTIC PATHWAYS**

**EXTRINSIC and INTRINSIC**

- **Death receptors** – Fas/TNFR
  - FasL/TNFα
  - Adaptor proteins: FADD/TRADD

- **DISC**
  - Procaspase 8 or 10

- **Apoptosome**
  - Caspase 8 or 10
  - Effector caspases: (3, 6, 7)

- **Cytochrome c**
  - Δψ
  - Apoptosis

- **IAP proteins**

- **Bcl-2**
- **nFκB**
- **p53**
- **c-myc**
- **Bad**
- **Bax**

**Stages of Apoptosis**

1. **Extrinsic Pathway**
   - FasL/TNFα activates death receptors.
   - Adaptor proteins (FADD/TRADD) recruit procaspase 8 or 10, forming a DISC.
   - Activated DISC leads to the recruitment of effector caspases.
   - Cytochrome c is released and forms the apoptosome with APAF-1.
   - Activated caspase 9 induces apoptosis.

2. **Intrinsic Pathway**
   - Bcl-2 family proteins regulate mitochondrial membrane permeability.
   - Cytochrome c released from mitochondria forms the apoptosome with APAF-1.
   - Activated caspase 9 induces apoptosis.

**Regulatory Proteins**

- **IAP proteins** block caspase activity.
- **nFκB** and **p53** regulate cell survival and death.

**Pathways**

- **FasL/TNFα** activates death receptors.
- **Bcl-2** family proteins modulate mitochondrial function.
- **nFκB** and **p53** regulate cellular responses.

**Conclusion**

Apoptosis is a complex process involving multiple pathways and regulatory proteins, ensuring controlled cell death in various biological contexts.
HEPATITIS C VIRUS

- Family Flaviviridae, enveloped virus
- Classification D.Baltimore → group IV: ssRNA(+) – positive-sense single stranded RNA
- 7 major genotypes, 67 subtypes, quasispecies

Envelope glycoproteins:
- E1 (gp31)
- E2 (gp70)

Core protein (p21)

Genome RNA

Envelope proteins
- Serin protease
- Ion channel
- NS4B protein

Core protein
- Cystein protease

Regulatory protein
- RNA-dependent RNA - polymerase
Pathophysiology of CHC

EXPOSURE
- Exposure to HCV
- Immune response CD4, CD8 T cells, interferon expression

APOPTOSIS
- Apoptosis in attempt to clear the virus (15-45% eradication)
- Immune evasion-lymphocytes apoptosis among different mechanisms
- Establishment of persistent infection

SEQUELAE
- Liver damage
- Cirrhosis
- Hepatocellular carcinoma (HCC)

Hajarizadeh B et al. Nat Rev Gastroenterol Hepatol. 2013 Sep;10(9):553-62
Role of HCV proteins to induce apoptosis

- HCV core protein: activates Fas, activates TRAIL, induces of mitochondrial stress (ROS, Cyt c release),
- NS3/4A complex: induces of mitochondrial stress (ROS, Cyt c release)
- NS5A: binds to protein kinase R leading to the inhibition of anti-apoptotic protein synthesis
- E1, E2: increase FasL expression

Role of HCV proteins to inhibit apoptosis

- HCV core protein: binds to c-myc, activates NF-κB, inhibits mitochondrial pathway
- NS3/4A: cleaves MAVS (Cardif) inhibiting intrinsic apoptotic pathway
- NS2: binds to CIDE-B inhibiting intrinsic/mitochondrial pathway
- NS5A: interacts with TRADD blocking caspase activation, activates NF-κB and protooncogen STAT-3 through triggering oxidative stress and ROS production; binds to proapoptotic protein Bax; inhibits proapoptotic protein Bid, activates protooncogen β-catenin, binds to p53 protein
- E1, E2: repress the activation of caspase-8 and the release of cytochrome c from the mitochondria

Role of apoptosis in pathogenesis of HCV-associated liver inflammation

Apoptosis plays a critical role in HCV-associated liver injury

Role of apoptosis in pathogenesis of HCV-associated liver inflammation

Detection of caspase-mediated CK-18 cleavage in HCV-infected liver biopsies.

Serum concentration of caspase-cleaved CK-18 epitop M30 and inflammatory activity in liver biopsies in CHC

Role of apoptosis in pathogenesis of HCV-associated hepatic fibrosis

Role of apoptosis in pathogenesis of HCV-associated liver injury

Hepatocyte Fas-expression

↑ LIVER INFLAMMATION

APOPTOSIS

Kupffer cell mediated phagocytosis of apoptotic bodies

NK cells T cells activation

IFN-γ

TGF-β1

Activated HSC

TGF-β1 Collagen type 1

↑ LIVER FIBROSIS

Malhi H et al. Physiol Rev. 2010; 90(3): 1165–1194
Carcinogenesis in CHC

- Direct:
  - HCV infection deregulates host cell cycle checkpoints
  - Immune- and virus-mediated oxidative stress and DNA damage
  - Infected cells accumulate mutations, eventually resulting in transformation

- Indirect:
  - Immune- or virus-mediated apoptosis
  - Compensatory proliferation and reinfection
  - Uninfected bystander cells accumulate mutations in an environment of inflammation and oxidative stress
  - Proliferation of transformed hepatocytes
Apoptosis of lymphocytes in CHC

Increased susceptibility to apoptosis

CD4, CD8 T-cells:
- ROS increased in PBMC in CHC
- TRAIL, Fas expression upregulated in NK, CD4, CD8 T-cells
- increased downstream caspase activity
- In HCV-specific CD8+ T-cells:
  1. increased PD-1 expression
  2. increased annexin V expression
  3. increased frequency of caspase 9-mediated T-cell death within the liver
  4. significant functional deficits – phenomenon of T-cells exhaustion

Adapted from:
Yi JS et al. Immunology. 2010;129(4): 474–81

Panasiuk et al. Liver Int. 2010;30(3):472-8
Stegmann KA et al. Gastroenterology. 2010;138:1885-97
Apoptosis of lymphocytes in CHC

Apoptosis of lymphocytes in CHC

Arends JE et al. Apoptosis. 2011 Sep; 16(9): 959–966

Patients n=8 (incl. placebo n=2), HC n=5


Patients n=7, HC n=7

*P<0.01

Panasiuk et al. Liver Int. 2010;30(3):472-8
Effects of DAA treatment on T cells population

- 51 previously untreated chronically infected patients undergoing IFN-free therapy with a combination of faldaprevir (NS3/4A protease inhibitor) and deleobuvir (a non-nucleoside NS5B inhibitor) with or without ribavirin
- SVR12 n=28, failure n=13
- prompt restoration of HCV-specific CD8+ T cell proliferation under successful IFN-free therapy
- increase HCV specific responses after \textit{in vitro} expansion per patient

Martin B et al. J Hepatol. 2014 Sep;61(3):538-43
Pancaspase inhibitors in HCV infection
Liver inflammation & fibrosis

- Oral emricasan (IDN-6556/PF-03491390), an antiapoptotic irreversible caspase inhibitor, lowers aminotransferase activity in patients with chronic hepatitis C
  - CHC n=80,
  - mean ALT decrease 22-56%
  - all HCV treatment groups above 5 mg QD were significantly different from placebo
  - HCV-RNA levels remained stable within 1 log unit during the study

Treatment with low-doses of GS-9450 resulted in lower ALT-values, but did not affect either the HCV viral load or the peripheral T-cells apoptosis rates (caspase 3, caspase 8, Fas expression)
The phenomenon of apoptosis in CHC is responsible for liver inflammation and fibrosis, is substantial for cancer formation and facilitate immune evasion.

DAA treatment allows to restore HCV-specific CD8 cells population and functionality of these cells (increased HCV specific responses and reduced expression of the markers of T cell exhaustion, e.g. PD-1) although further confirmation is necessary.

Therapeutic interventions in apoptotic signalling are limited by a complex nature of apoptosis in CHC and seem to be most applicable in the future in patients with advanced fibrosis / cirrhosis who previously completed successful antiviral treatment.