Apoptosis and Heart Disease

REVIEWS

Clerk et al. Regulation of cardiac myocyte death. *Pharmacol Ther* 2003; 97:223-61
Effects of myocardial infarction on the heart

Posterior wall of the LV: myocardial hypertrophy.
Anterior wall of the LV: scarring.

Myocyte death:

- Necrosis?
- Apoptosis?
- Other?
Cell death: necrosis vs apoptosis?

- Necrosis - Unregulated cell death associated with cellular disruption and an inflammatory response
- Apoptosis (programmed cell death) - Regulated cell death; controlled disassembly of cellular contents; no inflammatory response (e.g. hematopoietic cells)

2 ways to die?
Cell death:
Four deaths and a funeral?
( Nature Reviews: Molecular Cell Biology 2001 2: 1 - 10)

• Necrosis - Unregulated cell death associated with cellular disruption and an inflammatory response

• Apoptosis (programmed cell death) - Regulated cell death; controlled disassembly of cellular contents; no inflammatory response (e.g. haematopoietic cells)

• Apoptosis-like PCD - Some, but not all, features of apoptosis. Display of phagocytic recognition molecules before plasma membrane lysis

• Necrosis-like PCD - Variable features of apoptosis before cell lysis. “Aborted apoptosis”
Is there a graded response?

Apoptosis → Features of both → Necrosis

Mechanisms of cell death?
Apoptosis - The executioners

Caspases -
Cysteine-dependent aspartate-directed proteases

Executioners of apoptosis

Activated by proteolysis

(Other proteases may be involved - calpains, cathepsins)
Initiator caspases

Caspase 2
Caspase 9
CARD p20 p10

Caspase 10
Caspase 8
DED DED p20 p10

Effector caspases

Caspase 3
Caspase 6
Caspase 7
p20 p10

Other caspases

Caspase 1
Caspase 4
Caspase 5
Caspase 12
Caspase 13
CARD p20 p10

CARD (CAspase Recruitment Domain)
DED (Death Effector Domain)
Caspase cascades
Caspases execute the apoptotic programme

Inactivate proteins or complexes

Activate enzymes (e.g. protein kinases) by direct cleavage or cleavage of inhibitory molecules
Mechanisms of caspase activation

• Death by design - Receptor mediated pathways

• Death by default (neglect) - mitochondrial death pathway
The Death Receptors

Extracellular

Cysteine rich domains

Transmembrane domain

Death domain (DD)

Intracellular

TNFR1  Fas  DR3  DR4  DR5  DR6  NGFR

N  N  N  N  N  N  N
Components of receptor-mediated apoptosis: Adapter proteins

Death domain (DD)

Death effector domain (DED)
Signalling through death receptors

- Receptor trimerization
- Recruitment of adapter protein (FADD) through DD
- Recruitment and oligomerization of Caspase 8 through DED
Caspase 8 oligomerization results in cleavage and activation
Caspase 8 activates downstream caspases
Death receptor activation of caspase 8 is inhibited by FLIP

Caspase 8 homology, but no proteolytic activity
Flip inhibits Caspase 8 activation
Mitochondrial regulation of apoptosis

- Loss of mitochondrial membrane potential
- Release of cytochrome c
- Release of other apoptosis inducing factors
- Formation of the apoptosome complex (Cytochrome c, Apaf-1 (Apoptotic activating factor-1), dATP, Caspase 9)
Apoptosome formation
Oligomerization activates caspase 9 which initiates a caspase cascade.
Modulators of response:

- Inhibitor of apoptosis proteins (IAPs) (Inhibit caspase activity)
- Smac/Diablo - inhibit IAPs
- Heat shock proteins - HSPs
- Bcl-2 family proteins
Principal mechanisms of apoptosis

- FasL, TNF
- FADD
- Caspase 8
- Caspase 3
- Cytochrome c
- Apoptosome (Apaf1, caspase 9, ATP)
- Proteolysis
- Cellular stresses
- ROS
BUT…..

- Other proteases (calpains) may promote caspase-independent cell death particularly in relation to calcium.

- Energy levels in the cell may determine whether death is by necrosis or apoptosis (the apoptosome requires ATP).

- Oxidative stress inhibits the energy flux through the mitochondria, reducing ATP production.

![Diagram showing the relationship between apoptosis, necrosis, ATP, and ROS](diagram.png)
Bcl-2 family proteins

Anti-apoptotic  Pro-apoptotic
Bcl-2          Bad
Bcl-xL         Bax
(Mitochondrial)  Bcl-xS
(Cytosolic/Mitochondrial)
Function of Bcl-2 proteins?

1. Form ion channels in mitochondrial membrane
2. Interact with permeability transition pore (PTP)
3. Other?
A model for the regulation of apoptosis by Bcl-2 family proteins by heterodimerization/oligomerization
Bid links receptor and mitochondrial pathways

**Bid**

- **FasL, TNF**
- **FADD**
- **Caspase 8**
- **Caspase 3**
- **Cytochrome c**
- **Apoptosome** (Apaf1, caspase 9, ATP)
- **Proteolysis**

**Cellular stresses**

**ROS**
Features of apoptosis:

1. DNA fragmentation - DNA ladders
   TUNEL assay
Features of apoptosis:

2. Caspase 3 activation - Use antibodies to activated form for staining or western blots

3. Initiator caspase activation

4. Cytochrome c release - immunostaining, Western blots

5. Loss of mitochondrial membrane potential
   Fluorescent dyes for FACs or staining

6. Phosphatidylserine externalization (Annexin V stain)

7. Upregulation of death receptors
Apoptosis in the heart

• Required during embryonic development (modeling of the heart)


Death by design - probably receptor mediated

  (Caspase 8 -/- transgenics are embryonic lethal with severe cardiac abnormalities)
Apoptosis in human heart disease


1. End stage heart failure for transplants
   (cytosolic cytochrome c and increased caspase-3; Necrosis)

2. End stage heart failure + LV assist device
   (DNA fragmentation - ladders)

3. Ischemic heart failure
   (increased DNA fragmentation and Fas expression)

4. Myocarditis (soluble FasL)

5. End stage heart failure (DCM; IHD) (DNA fragmentation)
Apoptosis in animal models

1. Overexpression of G\(\alpha_q\), G\(\alpha_s\), caspase 8
2. Viral myocarditis
3. Ischemia/reperfusion (in vivo and ex vivo)
4. Pacing-induced dilated cardiomyopathy
Mechanisms involved in cardiac myocyte apoptosis

- FasL, TNF
- FADD
- Caspase 8
- Caspase 3
- Cytochrome c
- Apoptosome (Apaf1, caspase 9)
- Proteolysis

Cellular stresses
ROS
Receptors?
Mitochondria?
Both?
Death receptors do not always signal to myocyte apoptosis

Cardiospecific overexpression of FasL

No apoptosis

Hypertrophic gene expression

FasL, TNFα

Activation of other signalling pathways (e.g. MAPKs, NFκB)

Hypertrophy

Proteolysis

Caspase 8

FADD

FLIP

Caspase 3
Ischemia/reperfusion may sensitise myocytes to death receptor killing

Jeremias et al. 2000
Involvement of CD95/Apo1/Fas in cell death after myocardial ischemia.
Circulation 2000 Aug 22;102(8):915-20
Flip is downregulated during hypoxia

Myocyte apoptosis following I/R is reduced in lpr mice

Lpr = CD95 (Fas) -/-
Myocyte apoptosis through the mitochondrial pathway?

- FasL, TNF
- FADD
- Caspase 8
- Bid
- Caspase 3
- Cytochrome c
- Apoptosome (Apaf1, caspase 9)
- Proteolysis
- Cellular stresses
- ROS
- I/R
- Hypoxia
Oxidative stress-induced myocyte apoptosis ($\text{H}_2\text{O}_2$)

1. Markers for apoptosis
   (Caspase 3 and PARP proteolysis, TUNEL analysis)

2. Mitochondrial involvement
   (Cytochrome C release, Loss of mitochondrial membrane potential)

(Cook et al. 1999 Circ Res 85: 940-949)
Oxidative stress-induces DNA fragmentation (TUNEL)
Oxidative stress (0.5 mM H$_2$O$_2$) activates caspase 3 in neonatal myocytes
Oxidative stress (0.5 mM H$_2$O$_2$) induces cytochrome c release from the mitochondria in neonatal myocytes.
Oxidative stress (0.5 mM H$_2$O$_2$) promotes loss of ⚫ in neonatal myocytes
Hypoxia/reoxygenation induces cytochrome c release in adult myocytes
Hypoxia/reoxygenation activates caspases 9 and 3 in adult myocytes
Oxidative stress-induced myocyte apoptosis

1. Markers for apoptosis
   (Caspase 3 and PARP proteolysis, TUNEL analysis)

2. Mitochondrial involvement
   (Cytochrome C release, Loss of mitochondrial membrane potential)

3. Effects on Bcl-2 family proteins
   (Bcl-2, Bad, Bcl-xL, Bax)
Regulation of apoptosis by Bcl-2 family proteins by heterodimerization
Developmental regulation of Bcl-2 family proteins
Subcellular localisation of Bcl-2 proteins
Oxidative stress (0.5 mM H$_2$O$_2$) induces translocation of Bad to the mitochondria.
Oxidative stress (0.5 mM H$_2$O$_2$) promotes loss of Bcl-2 from the mitochondria
Bid links receptor and mitochondrial pathways

Calpain cleaves Bid during myocardial ischaemia/reperfusion
CARDIAC MYOCYTE

Growth signals

Cytoprotection

Hypertrophy/
Cell division

Death signals

Apoptosis
Cytoprotective pathways?

- Bcl-2, Bcl-xL
- FLIP, IAPs
- IGF-1, Insulin
- Phosphatidylinositol 3-kinase and protein kinase B (Akt)
- [Caspase inhibitors (may switch to necrosis)]
The phosphatidylinositol 3’ kinase (PI3K) pathway
IGF-1 and PI3K are cytoprotective

Serum-deprivation induced myocyte apoptosis
IGF-1 and Akt are cytoprotective
Akt is protective against ischaemia/reperfusion