Mitochondrial dynamics in ischemia and reperfusion

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I have nothing to disclose.
Novel therapeutic targets are required to protect the heart against ischemia-reperfusion injury (IRI).

Preventing mitochondrial dysfunction during IRI is an important strategy for cardioprotection.

Mitochondria can move and change their shape—‘mitochondrial dynamics’.

Mitochondrial dynamics as a novel target for protecting the heart against IRI.
Myocardial reperfusion injury

Yellon & Hausenloy, NEJM 2007
Ischemic preconditioning/postconditioning (Adenosine, Bradykinin, Opioids, EPO)

**Direct mPTP inhibition**
- Ras
- PI3K
- Raf
- Mek1/2
- Erk1/2
- Akt
- GSK3β
- PKCε
- Mito K ATP channel

**Indirect mPTP inhibition**
- eNOS
- NO
- PKG

**Mitochondrial dynamics**
- Less detrimental ROS
- Better ATP levels
- Acidosis at reperfusion
- Less calcium loading

**Cardioprotection**
The MPTP as a target for cardioprotection

Hausenloy et al 2002, 2003 CVR
Hausenloy et al 2004 AJP
Lim et al 2007 CVR
Selvanagam et al 2005 AJP
Piot et al 2008 NEJM
Mitochondrial fission and fusion

Fusion (Elongation)

Fusion proteins
- Mfn1
- Mfn2
- OPA1

Fission (Fragmentation)

Fission proteins
- Drp1
- hFis
- Mff
- MiD49/51

Mitochondrial Morphology

mPTP
Mitochondrial are dynamic organelles

Courtesy of Dr Sean M Davidson
Mitochondrial morphology in HL-1 cells

Fusion (Elongation)

Fission (Fragmentation)
Mitochondria fragment with ischaemia


Control cells

Drp1K38A transfected cells

Pre-ischemia

120 min ischemia

30 min reperfusion
Mitochondria fragment with ischaemia


Cells with elongated mitochondria (%)

Drp1K38A

Control

Start of hypoxia 2h hypoxia Start of reoxygenation 0.5h reoxygenation

Cells with elongated mitochondria (%)
Inhibiting mitochondrial fission
Inhibiting mitochondrial fission


![Image of mitochondrial fission](image)

**Diagram B:**
- **Vector Control**
- **Mfn1**
- **Mfn2**
- **Drp1K38A**
- **hFis1**
- **Vector Control + CsA**

**Normalized time until mPTP opening:**

- **Vector Control** (1.0)
- **Mfn1** (2.5)
- **Mfn2** (2.5)
- **Drp1K38A** (3.0)
- **hFis1** (2.0)
- **Vector Control + CsA** (1.0)

*Significance levels:* *p < 0.05, †p < 0.01*
Why do mitochondria fragment in response to ischemia?

- In non-cardiac cells it has been shown that calcineurin dephosphorylates Drp1 allowing it to translocate to mitochondria and induce fission.

  Cereghetti et al PNAS 2008

- Therefore, calcineurin inhibition prior to ischemia may protect against IRI by inhibiting mitochondrial fragmentation.
siRNA ablation of Mfn2 in neonatal cardiomyocytes induced mitochondrial fragmentation and increased susceptibility to mPTP opening and death.

Opposite findings of Mfn2 ablation in adult heart - see later.
Mitochondria are less mobile and are constrained between myofibrils. How relevant is mitochondrial dynamics to the adult heart?
Pharmacological inhibition of Drp1 cardioprotective in adult heart.

**Adult cardiomyocyte model of SIRI**

- mdiv-1
- Simulated Ischemia
- Simulated Reperfusion
- Cell viability determined

**In vivo murine MI model**

- mdiv-1
- Ischemia
- Reperfusion
- Infarct size determined

**Graphs:**

- **Cell death (%)**
  - Vehicle Control
  - 10µM mdiv-1
  - 50µM mdiv-1
  - 

- **Infarct / AAR (%)**
  - Vehicle Control
  - mdiv-1 (Dose 1)
  - mdiv-1 (Dose 2)
siRNA ablation of Drp1 in adult rat heart

Wang et al Nat Med 2011:17;71-79

- Genetic inhibition of Drp1 cardioprotective in adult rat heart
- miR-4199 upstream of calcineurin-Drp1-fission.
Mfn2 knockdown in the adult heart


- Mice deficient in cardiac Mfn2 have cardiac hypertrophy, large pleomorphic mitochondria, and are protected against mPTP opening and IRI (function). How does Mfn2 induce mPTP opening?
Mfn1 knockdown in the adult heart


- Mice deficient in cardiac Mfn1 have normal basal function, smaller mitochondria and are protected against ROS-induced mPTP opening.
- How does Mfn1 induce mPTP opening? Function under stress?
Mfn1 and Mfn2 double KO in adult heart

Chen et al Circ Res 2011:109;1327-1331

- Mice deficient in both cardiac Mfn1 and Mfn2 displayed mitochondrial fragmentation and dysfunction and developed cardiomyopathy.
- Mitochondrial fusion essential to the adult mammalian heart.
Mice partially deficient in OPA1 (+/-) had no basal phenotype, had large pleomorphic mitochondria, were protected against mPTP opening, but were more susceptible to hypertrophy (TAC).

How does OPA1 induce mPTP opening?
Parkinson's disease proteins and the heart

Mutations in DJ-1 cause mitochondrial dysfunction and Parkinson’s disease. Role in heart unknown.

Over-expressing DJ-1 causes mitochondrial elongation, delays MPTP opening and reduces cell death post-SIRI.
Adult mice deficient in DJ-1 have more fragmented mitochondria.
Adult mice deficient in DJ-1 have no cardiac phenotype on echocardiography but are more susceptible to IRI and are partially resistant to IPC. PD patients have more CV disease (36%) Jones et al Parkinsonism and Related Disorders 2012.
• MiD49/51 are outer membrane proteins of the fission machinery.
Conclusions

• Despite the unique arrangement of mitochondria in the adult heart, mitochondrial dynamics appears to be important for normal heart function.

• Inhibiting Drp1-mediated mitochondrial fission in the adult heart may be a novel therapeutic strategy for cardioprotection.

• hFis1, Mff, MiD49/51 are potential novel therapeutic targets for cardioprotection. Also DJ-1 protein.

• The function of the mitochondrial fusion proteins (Mfn1, Mfn2, OPA-1) in the adult heart is more complex and appears to be cell-specific.
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