Ischemic Protection in Cardiovascular Disease

- An Overview for Therapeutic Application -
• Is **Cardioprotection** from severe ischemia **possible**?

• What is the **adaptive behavior of the Cell** in protecting itself from ischemia?
Cardioprotection: Genesis of Concept

• Major paradigm shift by Braunwald et al (1971)
  
  Extent & severity of tissue damage after coronary occlusion
  
  : modified therapeutic manipulation during ischemia

• Experimental interventions for cardioprotection
  
  Exception of “early (timely) reperfusion”,
  
  none has been translated into clinical practice

• Considerations for effective clinical treatment

  Survive in any arrhythmia
  
  Minimize damaged functional myocardium
Ischemic Protection in Cardiovascular System

• Myocardial Response to Ischemic Injury
  
  Ischemic Preconditioning (PC)
  Hibernation
  Stunning

  Regulation of Ischemia-induced Oxidative Stress

• Therapeutic Strategy for Ischemic Protection
  
  Gene therapy
  Cell therapy: Angiogenesis vs Myocardial regeneration

• Future perspectives
  
  Considerations for Effective treatment
Ischemic Preconditioning

40' ischemia  3 days reperfusion

% infarction of risk zone

Delayed lethal cell injury in ischemic myocardium

(Murry et al Circulation. 1986;74:1124-1136)
Temporal Nature of 2 windows of Preconditioning

![Graph showing the degree of infarct size reduction over time in hours.](image)
Biphasic Pattern of Ischemic Preconditioning

- **Classic or Early phase of IPC**
  1-2hrs after PC stimuli

- **Late Phase of IPC** (Second Window)
  “Universal response of the heart to stress in general”
  12-72 hrs after PC stimuli

PKC-ε → NF-κB → iNOS / COX-2
Increased Bcl-2 expression → ↓ MPT opening
Primary Signaling Pathway in Preconditioning

End Effect of Cardioprotection: reduced cell death both necrosis & apoptosis
Secondary Signaling Pathway in Preconditioning

Converge to a few related mitochondrial proteins.

- ERK: phosphorylate mitochondrial-associated BAD which would cause it to dissociate from bcl-2, leaving bcl-2 free to bind and inhibit VDAC and the MPT.

- PKC, NO, 12-LO metabolites, ROS: activate the mito $K_{ATP}$ channel, reduce apoptosis and cell death.

- Mito $K_{ATP}$: maintain mitochondrial structure
  VDAC is in a low conductance state, synergize with the bcl-2
  VDAC in a closed state: reduce apoptosis
  (either via VDAC association with BAX or as part of the MPT).

- PC signaling : mito $K_{ATP}$, VDAC, and MPT
- Delayed PC: additional targets, such as NF-kB, iNOS, COX-2, p70s6K,
Mitochondrial Permeability Transition (MPT)

Mitochondrial Permeability Transition (MPT)

Opening of PMTP in the inner mitochondrial membrane
  → matrix swelling, outer membrane rupture
  → Release of apoptotic signaling molecules (cytochrome c )
  → Irreversible injury to the mitochondria.

During ischemia
  Intracellular Ca^{2+}, long-chain FA accumulation, ROS
  → ↑ mitochondrial susceptibility to MPT,
  → ↑ the likelihood that MPT will occur on reperfusion

Functional cardiac recovery : depends on mitochondrial recovery

**Cardioprotection by ischemic preconditioning**
**must ultimately involve the prevention of MPT.**
Mitochondrial Death Pathway

Death receptor
- Fas

DISC
- Death-inducing signal complex

Extracellular stimuli
- Broad spectrum of stress

Intracellular stimuli
- Intrinsin pathway
  - mitochondrial

Bcl-2 family
- Anti-apoptotic
  - Bcl-2, Bcl-XL

- Proapoptotic
  - Bax, Bak
  - Bid, Bad

APSF-1
- apoptotic protease activating factor-1

Caspase: cysteine protease
- executor of apoptosis
- cleave substrate

Caspase-3 activation
- Death

Mitochondrial Death Pathway: Apoptosis

Cytochrome C: apoptogenic mitochondrial protein

Crow MT et al. Cir Res. 2004;95:957-970
Trigger Mechanism of Early IPC

Role of Bradykinin at rabbit model

Pharmacologic PC
- Endotoxin, cytokine,
- ROS, NO donor,
- adenosin A1, A3 receptor agonist
- Endotoxin derivatives
- δ1-Opioid receptor agonist

Cellular Stress
- Sublethal ischemia
- heat stress
- Pacing
- exercise

(Goto et al Circ Res. 1995;77:611-621)
Mechanism of Late IPC
Cellular Mechanism of Late Ischemic Preconditioning

**Pharmacologic PC**
- Endotoxin, cytokine, ROS, No donor, adenosin A1, A3 receptor agonist
- Endotoxin derivatives
- δ1-Opioid receptor agonist

**Cellular Stress** (Sublethal ischemia, heat stress, Pacing, exercise)

**Trigger of Late PC**
- NO
- ROS
- Adenosine
- Opioid receptor agonist
- PKC (ε isoform)
- PTKs (Src/Lck)

**Activation of Kinases**
- MAPKs
- NF-κB
- AP-1

**Gene Transcription**
- iNOS
- Cox-2 (PGE2/PGI2)
- Aldose Reductase
- MnSOD
- HSPs
- PTKs (Src/Lck)
- MAPKs

**Mediator of Late PC**
- K<sub>ATP</sub> channels

**Universal response of the heart to stress in general**

**PROTECTION**
- Post translational modification
Hibernation and Stunning

- **Hibernation**: metabolic adaptation
  - first described by Rahimtoola
  - Loss of contractile fx to signaling by inflammatory-like process

- **Stunning** (prolonged post-ischemic ventricular dysfunction)
  - defined by Braunwald and Kloner
  - Oxygen free radicals (oxyradical hypothesis)
  - Cytosolic calcium overload (calcium hypothesis)

- **Ischemia-reperfusion Injury**
Mechanism of Ischemic Reperfusion Injury

- Ischemia and Reperfusion
  - Free Radicals
  - Ca$^{2+}$ Overload
  - Proteolysis of Tn-I, $\alpha$-actinin
  - Membrane damage
  - Contractile mechanism impaired

Relative Ca$^{2+}$ Insensitivity
Apoptosis: Ischemic Reperfusion Injury

Reperfusion: oxygen, glucose
Energy required
Completion of apoptosis
Acceleration of apoptosis
• Cardioprotection from severe ischemia is possible

• Adaptive behavior of the Cell in protecting itself from ischemia
  - not only cardiomyocytes -
Gaps in Knowledge That Hinder Translation

- Reproducibly effective in clinical relevant setting
  DM, HT, Hypercholesterolemia, LVH, old age

- Uncertainty regarding the magnitude of reperfusion injury

- Reliablity of methods to measure infarct-size

- Relevant model of sudden cardiac death

- Lack of appropraite biosensors in the setting of ischemia and cardiac arrest
Opportunities

- Infract size reduction is feasible
- Preconditioning
- Reliability of methods to measure infarct-size
- Progress in unraveling the mechanism of ischemia/reperfusion injury and protection
- Encouraging clinical data
  GIK, GUARDIAN, EXPEDITION
# Regulation of Oxidative Stress: Role of ROS

<table>
<thead>
<tr>
<th>ROS molecule</th>
<th>Main Sources</th>
<th>Enzymatic defense system</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide (O$_2^\cdot^-$)</td>
<td>‘Leakage’ of electron from the electron transport chain</td>
<td>Superoxide dismutase (SOD)</td>
<td>H$_2$O$_2$ + O$_2$</td>
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<tr>
<td></td>
<td>Activated phagocytes</td>
<td>Superoxide reductase</td>
<td>H$_2$O$_2$</td>
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<td></td>
<td>Xanthin oxidase</td>
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<td></td>
<td>Flavoenzyme</td>
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<tr>
<td>Hydrogen peroxide (H$_2$O$_2$)</td>
<td>From O$_2^\cdot^-$ via SOD</td>
<td>Glutathion peroxidase</td>
<td>H$_2$O + GSSG</td>
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<tr>
<td></td>
<td>NADPH-oxidase</td>
<td>Catalsae</td>
<td>H$_2$O + O$_2$</td>
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<td>Glucose oxidase</td>
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<td></td>
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<tr>
<td></td>
<td>Xanthin oxidase</td>
<td>Peroxiredoxin (Prx)</td>
<td>H$_2$O</td>
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<tr>
<td>Hydroxyl radical (·OH)</td>
<td>From O$_2^\cdot^-$ and H$_2$O$_2$ via transition metals (Fe,Cu)</td>
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<tr>
<td>Nitric Oxide(NO)</td>
<td>Nitric oxide synthases</td>
<td>Glutathion/ TrxR</td>
<td>GSNO</td>
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</tbody>
</table>
Redox Sensitive Signaling Pathway in Vascular Cell

Ang II, Thrombin, TNF-α, IL-1, PAF, Stretch, shear

Proximal receptors
- PLCγ, PLCβ, PLD
- Ca^{2+}, DG, AA
- PKC, Rac 1/2

Ca^{2+}

ROS

NAD(P)H oxidase

ROS

MAP Kinase
- p38 MAPK
- Akt/ PKB
- JNK/ SAPK
- Big MAPK, ERKS

Transactivated PDGF, EGF receptors
- SNC
- Grb2
- SOS

p42/44/ Erk 1/2 (variable oxidant sensitivity)

Kinases
- p70S6K, p90 Rsk

Protein synthesis

Redox-sensitive genes

Transcription Factors
- ATF-2, CHOP-1, AP-1, NF_{κ}b, Elk-1, E2F, STAT1

Hypertrophy, Migration, Proliferation
Endothelial dysfunction
Inflammation
## Redox Sensitivity of Gene Expression in Cardiovascular Cells

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cell Type</th>
<th>Stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCAM-1</td>
<td>Endothelial cells</td>
<td>TNF-α, IL-1α, IL-1β, IL-4</td>
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<tr>
<td>ICAM-1</td>
<td>Endothelial cells</td>
<td>TNF-α, NO, leukotrienes</td>
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<td>E-selectin</td>
<td>Endothelial cells</td>
<td>IL-1α, LPS, PMA, TNF-α</td>
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<tr>
<td>MCP-1</td>
<td>VSMCs</td>
<td>TNF-α</td>
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<td></td>
<td>VSMCs</td>
<td>PDGF</td>
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<tr>
<td>M6SF</td>
<td>Endothelial cells</td>
<td>TNF-α, ox-LDL</td>
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<td>H₂O₂, TNF-α</td>
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<td>Xanthine/xanthine oxidase</td>
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<td>nNOS</td>
<td>Endothelial cells</td>
<td>IL-1β</td>
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<td>Cu/Zn SOD</td>
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<td>H₂O₂</td>
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<td>Catalase</td>
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<td>H₂O₂</td>
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<td>Glutathione peroxidase</td>
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<tr>
<td>Mn-SOD</td>
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<tr>
<td>HU-1</td>
<td>Endothelial cells</td>
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<td></td>
<td>Macrophages</td>
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<td></td>
<td>VSMCs</td>
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<tr>
<td>Gox-2</td>
<td>Vascular smooth muscle cells</td>
<td>IL-1β</td>
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<tr>
<td>HSP-70</td>
<td>VSMCs</td>
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<tr>
<td></td>
<td>Endothelial cells</td>
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<tr>
<td>Serringer receptor</td>
<td>VSMCs</td>
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<tr>
<td>IL-8</td>
<td>Macrophages</td>
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<td>Microvascular smooth muscle cells</td>
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<tr>
<td>HB-ESF</td>
<td>Endothelial cells</td>
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<td></td>
<td>VSMCs</td>
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<tr>
<td>Atrial natriuretic factor</td>
<td>cardiac myocytes</td>
<td>PMA, H₂O₂/Vasodilat</td>
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<tr>
<td>VEGF</td>
<td>Endothelial cells</td>
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<tr>
<td></td>
<td>VSMCs</td>
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</tbody>
</table>

(Griendling KK et al ATVB. 2000;20:2175-2183)
# Targets for gene-based therapy for myocardial protection and rescue from ischemia induced Injury

<table>
<thead>
<tr>
<th>Strategy/ Therapeutic target</th>
<th>Genetic Manipulation</th>
<th>Vector</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protection/ Prevention</td>
<td></td>
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<tr>
<td>Anti-oxidant genes</td>
<td>HO-1, SOD, Catalase, GPX</td>
<td>Overexpression</td>
<td>ADV, AAV, LV, α-virus</td>
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<tr>
<td>Heat shock proteins</td>
<td>HSP70, HSP90,HSP27</td>
<td>Overexpression</td>
<td>ADV, AAV, LV, α-virus</td>
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<tr>
<td>Survival genes</td>
<td>Bcl-2, Akt, HGF</td>
<td>Overexpression</td>
<td>ADV, AAV, LV, α-virus</td>
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<tr>
<td>Inflammatory cytokines</td>
<td>ICAM,VCAM,TNF-α,NF-κB</td>
<td>Inhibition</td>
<td>AS-ODN, Decoy ODN, ADV-AS-ODN, RV-AS-ODN</td>
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<td>Adhesion molecules</td>
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<tr>
<td>Tissue Factors</td>
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<tr>
<td>Proapoptotic genes</td>
<td>Bad, P53, Fas ligand</td>
<td>Inhibition</td>
<td>AS-ODN, Decoy ODN, ADV-AS-ODN</td>
</tr>
<tr>
<td>Coronary vessel tone</td>
<td>e-NOS, adenosin receptors</td>
<td>Overexpression</td>
<td>RV, ADV, AAV(?)</td>
</tr>
<tr>
<td>Rescue</td>
<td></td>
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<tr>
<td>Proangiogenic gene</td>
<td>VEGF&lt;sub&gt;121&lt;/sub&gt;, VEGF&lt;sub&gt;165&lt;/sub&gt;, FGF-1, FGF-2, FGF-4, FGF-5, HGF, eNOS</td>
<td>Overexpression</td>
<td>Plasmid, ADV, AAV, LV (?)</td>
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<tr>
<td></td>
<td>Ang-1, MCP-1, G-CSF, PDGF-BB, IGF-1, IGF-2, HIF-1α</td>
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</tbody>
</table>

Therapeutic Strategy For Ischemic Protection

- Ischemic Protection by Gene Therapy

  Gene therapy: VEGF for ischemic limb.

  Heart Failure: target gene (3 biologic different pathway)

  1. Ca²⁺ homeostasis: Sarcoplasmic reticulum Ca²⁺-ATPase (SERCA 2A)
  2. β-adrenergic receptor: β-adrenergic receptor kinase 1 (βARK1)
  3. Reducing apoptosis: Bax, Bcl-2, Akt, p53 etc

  Redox sensitive gene.
Target gene of Heart Failure

• Ca^{2+} homeostasis: Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA 2a)
  
  SERCA (115 kDa): ↑ Ca^{2+} uptake to SR, ↓ Cytosolic Ca^{2+}

  Reduced expression or activity of SERCA 2a at failing heart

  Phospholamban: endogenous inhibitor of SERCA 2a

• β-adrenergic receptor: β-adrenergic receptor kinase 1 (β-ARK1)
  
  Down regulation of β-AR, Up regulation of β-AR Kinase at CHF

• Reducing apoptosis: Bax, Bcl-2, Akt, p53
Myocardial Gene Therapy

Role of Akt Signaling in Vascular Homeogenesis

 Akt (inactive)

 Akt (active)

Cell survival
Bad
Forkhead
IKKa
FLIP

Cell cycle
E2F
P21
MDM2
hTERT

Glucose metabolism
GSK3
GLUT4

Protein synthesis
mTOR
S6K1
4E-BP1

Growth factor-dependent survival pathway

Attachment-dependent survival pathway

(Shiroma et al Cir res. 2002:90:1234-1250)
Regenerative Medicine

Functional loss = disease

Medical approach
- drug
- Intervention, Surgery

Organ Transplantation

Regeneration of Organ

Cell Therapy: stem cell

Organ damage

Ischemic heart disease
Major Advances in Real World of Cardiology

[1] Intervention: Drug-eluting stent (DES)

[2] Cell (Stem cell) therapy in human trial
   - TACT study (2001), BM-derived MNCs local injection
   - TOPCARE-AMI, BM-derived MNCs intracoronary injection (2002)
     - TOPCARE-CHF
   - MAGIC-cell (2003)
   - BOOST trial (2004)
   - AC 133(+) cell intracoronary injection (2003)
   - Skeletal myoblast transendocardial injection
     - transvenous myocardial injection
Which patient should be considered for cell therapy?

Which type of stem cell should be used?

Which quantity and concentration should be used?

By what mechanism do stem cell engraft, survive, differentiate?

- Improvement: active (by increasing contractility)
  - passive (by limiting infarction and remodeling)

- Life span of transplanted stem cell

- Safe (long-term safety)

- Potential tumorigenesis

- Potential benefit in non-ischemic heart failure
Stem Cell Therapy

- Decreased Infarct Expansion
- Angiogenesis
- Myocardial Preservation
- Decreased Apoptosis
- Myocyte Regeneration
- Increased Collagen Expression
- Myocardial Regeneration

Attenuation or Reversal of Post-ischemic Damage
Stem Cell Therapy

Myocardial Regeneration

Myocardial Preservation

Decreased Infarct Expansion

Angiogenesis

Myocyte Regeneration

Decreased Apoptosis

Increased Collagen Expression

Attenuation or Reversal of Post-ischemic Damage

Functional Augmentation: Ex vivo modification
Future Perspectives

Genetic Modification

Adv-GSK, Ex Vivo modification

Mobilization

Homing
Plasticity of Adult Stem Cell

Possible Mechanism of the Plasticity

1. [Diagram of multiple stem cells]

2. [Diagram of cell fusion: (4n: 2n+2n)]

3. [Diagram of trans-de-differentiation or re-differentiation]

4. [Diagram of pluripotency: True pleuripotent or multipotent]

Homing and Differentiation of EPC

(Urbich and Dimmeler et al, Circ Res. 2004;95:343-353.)
Mobilization of EPC from Bone Marrow

- Activation of (MMP-9)
- mKitL to a soluble Kit ligand (sKitL)
- cKit-(+) stem and progenitor cells (hemangioblast, HABL), move to the vascular zone of BM
- from a quiescent to a proliferative state

- Early EPCs: CD133/CD34/VEGFR-2.
- Circulating EPCs: CD34/VEGFR-2/CD31/VE-cadherin/vWF

(Hristov et al, Ateriosclero Thrombo Vasc Biol. 2003;23)
Candidates for Mobilization

• SCF, c-kit and MMP-9
  - Role for stem cell mobilization after MI

• SDF-1 and CXCR-4
  - Migration of CD 34+ cells

• G-CSF
  - Via SDF-1 and CXCR-4

• VEGF and Flk-1
  - Angiogenesis
Myocardial Regeneration

Regeneration in the brain, spinal cord, intestine, heart, limb, lens & retina.

**Blastema**: collection of dedifferentiated cells at injury site

*phosphorylation of the proteins of retinoblastoma tumor suppress gene*

*Limited supply of diagnostic antibody*  
Natural Repair of the Heart

- **Traditional concept of the cardiomyocytes (CMCs)**
  - terminally differentiated cells
  - number of CMCs at birth only decrease with age
  - no house-keeping mechanism to repair any damage

  only Hypertrophy rather than hyperplasia

- **Improved LV function after MI**
  - Process of remodeling that
    - combination of hypertrophy and fibrosis
Myocardial Regeneration

• Human Cardiomyocytes divide after MI. Beltrami et al. NEJM 2001

10–60 fold increase in mitotic figures was recorded in patients dying from heart failure,

The mitotic proportion was low, 0.015-0.08%

? Act as an effective repair mechanism.
? The source of dividing Cell: unclear

• Chimerism of Transplanted Heart Quaini et al. NEJM 2002

Derived from Extra-cardiac Origin

2 potential sources
Bone-marrow
Residual cardiac stem cell

Male recipient + female donor Heart
Candidate Cells of Myocardial Regeneration

Autologous cells

- Differentiated cells
  - Skeletal muscle cells
  - Cardiomyocytes
  - Fibroblasts...

Endothelial progenitor cells

Skeletal myoblasts

Stem cells
- BM hematopoietic or mesenchymal stem cells
- Peripheral blood stem cells

Allogenic cells

- Stem cells from umbilical cord blood
- Embryonic stem cells
Questions?

• ? Possible origin of proliferating cells

• ? Clinical Significance

• ? Safety
  Functional and electrical integration
    : hypocontractile and proarrhythmic consequences

implanted stem cell
  ? differentiated to fibroblast: increased scar
  ? differentiated to myocyte
    discordance b/w structural contractile property
Human Skeletal Myoblasts and BM-derived CD133 Progenitors for the Repair of Infarcted Myocardium

Oxidative Stress

Cytokine-like effect, Chemokine-like effect

NADPH

Trx reductase

Trx-S₂ (oxidized)

Trx-(SH)₂ (reduced)

Protein-(SH)₂ (reduced)

Protein-S₂ (oxidized)

Prx-(SH)₂

PrX-S₂

H₂O₂

H₂O + O₂

Scavenger of Reactive Oxygen Species (ROS)

Cell growth

Inhibit apoptosis (ASK-1 ↓)

Transcription factors (NF-κB, AP-1)

Gene transcription

Inhibit inflammation

Inhibit apoptosis

Inhibit inflammation

Gene transcription
Plasma Thioredoxin (ng/ml)

- Control (n=15)
- VA (n=6)
- UA (n=21)
- NSTEMI (n=13)
- STEMI (n=27)

\[ (N=67) \]

\[ \text{WBC (mg/dl)} \]

\[ \text{Trx (ng/ml)} \]

\[ P=0.003 \]
§ 3 types of MI model

Coronary artery (LAD) ligation in Sprague-Dawley rats (N=71, n=3 at each time point)
- Sequential analysis: Control (normal), 0 hr, 30 min, 1hr, 2hr, 4hr, 12hr, 24hr, 48hr

- Non-reperfused transmural MI
  - MI -

- Early (45 min)-reperfused nontransmural MI
  - EMI -

- Late (5 hrs)-reperfused transmural MI
  - LMI -

Myocardial infarction
ROS overproduction

Myocardial injury
LV remodeling

Protein extraction for Western blot analysis
Expression of Myocardial Trx and TrxR in 3 MI models

<table>
<thead>
<tr>
<th>MI  (Non-Reperfusion infarction)</th>
<th>EMI (Early-Reperfusion infarction)</th>
<th>LMI (Late-Reperfusion infarction)</th>
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</thead>
<tbody>
<tr>
<td>Con 0h 0.5h 1h 2h 4h 12h 24h 48h</td>
<td>Con 0h 0.5h 1h 2h 4h 12h 24h 48h</td>
<td>Con 0h 0.5h 1h 2h 4h 12h 24h 48h</td>
</tr>
</tbody>
</table>

Trx  
TrxR  
SOD  
Actin

Graph showing changes in Trx, TrxR, and SOD over time for different MI models.
Immunohistochemistry for Trx localization

- Trx expression in infarct border-zone

Trx = Red Fluorescence
§ Hypoxic injury by anaerobic chamber

**HUVECs** (Human Umbilical Vein Endothelial Cells)
**EPCs** (Endothelial Progenitor Cells)
**VSMCs** (Vascular Smooth Muscle Cells)

Hypoxic injury (1% O₂)
0h, 0.5 hr, 1hr, 2hr, 4hr, 12hr, 24hr

Cell harvest

Protein Extraction for Western blot analysis
Hypoxic injury of 3 cell types (vascular cell)

- EPC
- HUVEC
- vSMC

<table>
<thead>
<tr>
<th></th>
<th>Con 0.5h</th>
<th>1h</th>
<th>2h</th>
<th>4h</th>
<th>12h</th>
<th>24h</th>
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<td>Tubulin</td>
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</table>

- con, 30min, 1h, 2h, 4h, 12h, 24h

- HUVEC
- EPC
- vSMC
Expression of Trx and Its downstream pathway in Balloon injury models

Trx (12kDa) control 4 days 2 weeks

NF \( \kappa B \) (65kDa) control 4 days 2 weeks

I \( \kappa B \) (37kDa) control 4 days 2 weeks
Expression of Trx and TrxR in TAC model

<table>
<thead>
<tr>
<th>Tying</th>
<th>Untying</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con 1D 3D 1W 2W 1D 3D 5D 1W 1W 2W</td>
<td></td>
</tr>
</tbody>
</table>

Trx

TrxR

SOD

Actin
eNOS production at 293 T cells and NO secretion VSMCs

- SHR - 6wks old male, 2 group. Lenti-eNOS virus vs Control (1x TBS injection)
  
  Receiving L-arginine hydrochloride (35.6mmol/L) in drinking water

**eNOS production at 293 T cells**

![Image]

**Marker**  
**Control**  
**293T**

**NO Concentration in supernatant**

- Rat aorta VSMCs only 0.00 pg/ml
- ecNOS transduced 293T cells 0.25 pg/ml
- ecNOS transduced rat aorta VSMCs 0.66 pg/ml
Serum NO levels after NOS gene therapy

- ecNOS transduced 293T cells
- Rat aorta VSMCs only
- ecNOS transduced rat aorta VSMCs

Nitrite (pg/ml)
GFP expression (*in vivo*)

<table>
<thead>
<tr>
<th>Maker</th>
<th>S.M</th>
<th>Bone</th>
<th>Brain</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Lung</th>
<th>Heart</th>
</tr>
</thead>
</table>

[Image of GFP expression in various tissues]
Co-polymerization of PGCL

Glycolide (50%) + e-Caprolactone (50%)

Sn-oct
170 °C, 20 hrs

Glycolide/ e-Caprolactone Copolymer (PGCL)
Absorbed within 2 months
rMSCs Seeded on a PGCL Scaffold

Culture for 48hrs

H&E x 200

SEM x 1,100
rMSCs-Seeded PGCL Patch Implanted to Normal Heart

5 weeks after patch implantation

PGCL patch
Epicardium (EpiC)

Myocardium (MyoC)
rMSCs-Seeded PGCL Patch Implanted to Normal Heart

**DAPI-Labeling**

1 week after patch implantation

5 weeks after patch implantation

(My x200)
HUVECs and EPCs for Myocardial Regeneration

- Lentivirus-mediated GFP transfer to EPCs
- DAPI-labeled EPCs injection into normal myocardium

- Genetic makeup with VEGF, Akt, antiapoptotic genes
HUVEC: Co-culture with cardiomyocytes for 2 Weeks

Differentiation of HUVECs into a Cardiomyocyte Phenotype

BrdU (+) / Tn I (+) cell

HUVECs: pre-labeled with BrdU

Green fluorescence : BrdU   Red fluorescence : Troponin I (TnI)
In Vivo Study at Normal Myocardium

4 weeks after HUVECs injection into normal myocardium
Gross Findings After HUVECs transplantation

- 2 weeks after HUVECs injection into border zone

<table>
<thead>
<tr>
<th>Control (media only)</th>
<th>HUVECs transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masson trichrome staining</td>
<td>H&amp;E staining</td>
</tr>
</tbody>
</table>

[Images of Masson trichrome staining and H&E staining for both control and HUVECs transplantation samples]
DAPI (+) HUVECs at Both Infarct & Border zones

- 2 weeks after HUVECs injection into border zone
- DAPI labeled cells in border zone and infarct zone
HUVECs at Both Infarct & Border zones

DAPI (+) cells in Border zone and infarcted zone

DAPI (4',6-diamidino-2-phenylindole) labeling for 60 min
Cardiomyogenic Differentiation of Transplanted HUVECs or hEPCs into Infarct Border Zone

- Red: Cardiomyocyte maker (cTnI)
- Blue: Nuclei of transplanted HUVECs or hEPCs (DAPI)
Cardiomyogenic Differentiation of Transplanted HUVECs or hEPCs into Infarct Border Zone

- Red: Cardiomyocyte maker (Myosin heavy chain, MHC)
- Blue: Nuclei of transplanted HUVECs or hEPCs (DAPI)
Primitive Vessel Formation or Angiogenesis of Transplanted HUVECs or hEPCs

- Red: Endothelial marker (vWF)
- Blue: Nuclei of transplanted HUVECs or hEPCs (DAPI)
Gap Junction Formation of Transplanted HUVECs or hEPCs with Native Cardiomyocytes

• Red: Gap junction maker (Connexin 43)
• Blue: Nuclei of transplanted HUVECs or hEPCs (DAPI)