My Regeneration: Using Stem Cells to Repair the Heart

Community Medical School Spring 2013
Case

- 76 year-old man
- Chest pain at home
- 911 called (wife)
- Cardiac arrest on transport to ED
  - Sudden cardiac death ➔ defibrillated
- ED diagnosis
  - Acute myocardial infarction
- Emergency cardiac catheterization
  - Stent to the left anterior descending coronary artery
Case

- Large myocardial infarction (heart attack)
  - Cardiogenic shock
  - 2 months in the hospital
  - Miraculous survival
  - But, he had heart failure the rest of his life
- Lived ~2.5 more years
  - Both he and his family were grateful
My Regeneration: Using Stem Cells to Repair the Heart

- Normal heart
  - Anatomy and physiology

- Heart attack
  - Myocardial infarction
  - Sudden cardiac death

- Cardiac regeneration
The heart, who cares?

Table B. Deaths and death rates for 2011

<table>
<thead>
<tr>
<th>Cause</th>
<th>Deaths (in thousands)</th>
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</thead>
<tbody>
<tr>
<td>All causes</td>
<td>2,512,873</td>
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<tr>
<td>Diseases of heart</td>
<td>596,339</td>
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<tr>
<td>Malignant neoplasms</td>
<td>575,313</td>
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<tr>
<td>Chronic lower respiratory diseases</td>
<td>143,382</td>
</tr>
<tr>
<td>Cerebrovascular diseases</td>
<td>128,931</td>
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<tr>
<td>Accidents (unintentional injuries)</td>
<td>122,777</td>
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<tr>
<td>Alzheimer’s disease</td>
<td>84,691</td>
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<tr>
<td>Diabetes mellitus</td>
<td>73,282</td>
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<tr>
<td>Influenza and pneumonia</td>
<td>53,667</td>
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<tr>
<td>Nephritis, nephrotic syndrome and nephrosis</td>
<td>5</td>
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<tr>
<td>Intentional self-harm (suicide)</td>
<td>45,731</td>
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<tr>
<td>Septicemia</td>
<td>35,539</td>
</tr>
<tr>
<td>Chronic liver disease and cirrhosis</td>
<td>33,539</td>
</tr>
<tr>
<td>Essential hypertension and hypertensive renal disease</td>
<td>27,477</td>
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<tr>
<td>Parkinson’s disease</td>
<td>23,107</td>
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<tr>
<td>Pneumonitis due to solids and liquids</td>
<td>18,090</td>
</tr>
<tr>
<td>All other causes</td>
<td>512,723</td>
</tr>
</tbody>
</table>
What is the heart, and what does it do?

Anatomy and Physiology

Structure  Function
Anatomy of the Heart

Left side, right side, 4 chambers and 4 valves
Normal Anatomy and Physiology

The heart is a muscular pump
Normal Anatomy and Physiology

*The heart is a muscular pump*
Normal Anatomy and Physiology

Red blood cells and oxygen delivery
Normal Anatomy and Physiology

Valves → unidirectional flow of blood
Normal Anatomy and Physiology

Mitral Valve
Abnormal Mitral Valve
Anatomy and Physiology
Coronary Arteries
Anatomy and Physiology

“Coronary Arteries”
Coronary Atherosclerosis
“Cholesterol blockage”
What is a Heart Attack?

“Myocardial Infarction”
What is a Heart Attack?
“Myocardial Infarction”
Myocardial Infarction
Emergency Treatment
Myocardial Infarction/Heart Attack

Manifestations

Cell death (necrosis) because of lack of oxygen

Ventricular fibrillation

Sudden cardiac death
Myocardial Infarction/Heart Attack

Manifestations

Cell death (necrosis) because of lack of oxygen

Fibrosis
Myocardial Infarction/Heart Attack

Manifestations
My Regeneration: Using Stem Cells to Repair the Heart

- Normal heart
  - Anatomy and physiology
- Heart attack
  - Myocardial infarction
  - Sudden cardiac death
- Cardiac regeneration
My regeneration: Using stem cells to repair the heart

Jeff Spees, Ph.D.

Department of Medicine,
Cardiovascular Research Institute,
Stem Cell Core

University of Vermont
Goal:
To provide cardiac regeneration, improve cardiac function, and increase life-span in patients with MI.

Cardiovascular disease:
The number 1 killer of men and women.
Approximately 1.2M heart attacks/year. Over 400,000 deaths related to MI.
The cost of cardiovascular disease and stroke is >$300B/year.

Currently, there are no treatments that rescue or replace jeopardized or necrotic tissue after acute myocardial infarction (MI).
Two major issues for use of stem/progenitor cells for cardiac repair after acute myocardial infarction (MI)

1. Source of reparative cells.

   **Great progress:** Adult stem/progenitor cells or ES cell derivatives

2. Graft success.

   A. Adhesion
   B. Survival
   C. Migration
   D. Differentiation
   E. Functional integration into host cardiac tissue

   **Little progress:** Poor graft success is now the greatest challenge facing cell therapy.
Poor cardiac graft success with human ES cell derivatives

Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts
Poor cardiac graft success with human ES cell derivatives

Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts.
Why stromal cells may be of use to promote graft success:

Mesenchymal cells commonly found in connective tissue, vascular tissue, and stem cell niches provide several forms of support to surrounding cells (e.g. substrate, growth factors, cytokines).

Examples:

Fibroblasts, fibrocytes, pericytes, bone marrow MSCs
Stromal support cells of the bone marrow (MSCs)
MSCs:

“Mesenchymal Stem Cells” or “Multipotent Stromal Cells”

Non-hematopoietic (non-blood forming) bone marrow progenitor cells that can be isolated from bone marrow by adherence to tissue culture plastic or by MACS (e.g. p75LNGFR, CD271; or Prominin 1, CD133)

Rescue and repair tissues by:

1. Direct cell differentiation (bone, fat, cartilage, skeletal muscle, vascular endothelium and smooth muscle; other cell types?)

2. Cell fusion

3. Transfer of mitochondria

4. Paracrine action (secreted factors: growth factors, cytokines)

5. Stimulation of endogenous tissue stem/progenitor cells
Stem/progenitor cells from rat heart and human bone marrow

CSCs

CPCs

MSCs

p75MSCs

p75 Osteo

p75 Adipo
Stromal cell CdM induces proliferation of CPCs, but not cardiac fibroblasts

Cell number ratio to baseline (%) vs (Days)

CdM
- MSC donor 1
- MSC donor 2
- p75MSC donor 1
- p75MSC donor 2
- fibro donor 1
- fibro donor 2
- SFM

SFM
- MSC donor 1
- MSC donor 2
- p75MSC donor 1
- p75MSC donor 2
- fibro donor 1
- fibro donor 2

Cell number ratio to baseline (%) vs (Days)

Stromal cell CdM induces proliferation of CPCs, but not cardiac fibroblasts
Dose-responsive effects of MSC CdM on proliferation of CPCs

Cell number ratio to baseline (%)

(Days)

10x concentrated CdM
- MSC donor 3
- MSC donor 4
- p75MSC donor 1
- p75MSC donor 3

unconcentrated (1x) CdM
- MSC donor 1
- p75MSC donor 1
Stromal cells secrete ligands that induce P-STAT3 and P-Akt in CPCs

A

<table>
<thead>
<tr>
<th>SFM</th>
<th>M</th>
<th>p75</th>
<th>fib</th>
<th>SFM</th>
<th>M</th>
<th>p75</th>
<th>fib</th>
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<tbody>
<tr>
<td>p-STAT3</td>
<td></td>
<td></td>
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<tr>
<td>T-STAT3</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>β-ACTIN</td>
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</table>

Day 1

Day 2

B

C

<table>
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<tr>
<th>SFM</th>
<th>M</th>
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<td>p-Akt</td>
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<tr>
<td>T-Akt</td>
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</table>

Day 1

Day 2
Specific inhibition of STAT3 signaling by “Stattic” prevents CPC proliferation
Stromal cell CdM induces protects CPCs against 48hrs of simulated ischemia (1% oxygen) via STAT3 signaling.
CPCs remain multipotent after incubation in CdM from p75MSCs.
Myocardial infarction surgery
Myocardial infarction surgery

Before Injection | After Injection
No Ligation

Before Injection | After Injection
1 H Persistent Ligation

500 μL Evan’s Blue Dye (1%) Injection
Myocardial infarction surgery

Control

Permanent ligation (1 wk)
New methods of cell administration:

Tangential, sub-epicardial, border zone injections of CSCs after MI
Engraftment results at 1 week after MI and treatment with CSCs
Engraftment results at 1 week after MI and treatment with CSCs
Migration of CPCs to distal injured sites after 1 week
Engraftment results at 1 week after MI and treatment with CSCs
Microarray analyses of gene expression in freshly-isolated and cultured human bone marrow stem and progenitor cells
Antibody neutralization of CTGF blocks CdM protection of CPCs in hypoxia

(After transcriptional profiling of human p75MSCs (cDNA microarray assays), ELISAs of secreted peptides/proteins in CdM, neutralization assays of candidates).

Note: Insulin was identified in CdM by ELISA and blocked in CPC protection assays.
Structural domains of CTGF (a.k.a. CCN2, IGFBP8)

C-terminal domain (CTGF-D4) promotes cell adhesion, proliferation, and migration.
Incubation of CPCs in C terminal CTGF peptide (D4) induces P-STAT3
Incubation of CPCs in Insulin induces P-Akt

<table>
<thead>
<tr>
<th>Insulin (ng/ml)</th>
<th>0.1</th>
<th>1</th>
<th>50</th>
<th>100</th>
</tr>
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<tr>
<td>60 kDa</td>
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<tr>
<td>37 kDa</td>
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P-Akt

GAPDH
Synergistic effects of CTGF (C-term domain 4; CTGF-D4) and Insulin on protection of CPCs during exposure to hypoxia (1% oxygen)
“Cell-Kro”: a new tool to promote graft success

What is Cell-Kro?
Cell-Kro is a defined combination of human peptides derived from Connective Tissue Growth Factor (C-terminal, domain 4; CTGF-D4) and Insulin (alpha/beta peptide).

How does it work?
Cell-Kro acts like a “ligand backpack” to boost graft success after cell transplantation. Short-term (30 min) priming with Cell-Kro dramatically increases cell adhesion, proliferation, survival, and migration.

[Diagram showing the effect of Cell-Kro on cell behavior before and after priming.]
At 1 week after MI and treatment, graft success with CSCs primed in Cell-Kro was similar to that obtained after priming in p75MSC CdM

<table>
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<tr>
<th>Priming condition</th>
<th>Number of animals with successful engraftment after 1 week</th>
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</thead>
<tbody>
<tr>
<td>1% BSA in αMEM (Vehicle control)</td>
<td>1/7</td>
</tr>
<tr>
<td>Cell-Kro</td>
<td>5/5</td>
</tr>
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Engraftment results at 1 month after MI and treatment with CSCs

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<tbody>
<tr>
<td>1% BSA in αMEM (Vehicle control)</td>
<td>1/7</td>
</tr>
<tr>
<td>30x p75MSC CdM</td>
<td>4/4</td>
</tr>
<tr>
<td>Cell-Kro</td>
<td>4/5</td>
</tr>
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Cellular reprogramming technologies may provide improved cell-based therapy for myocardial infarction.
Induced Pluripotent Stem (iPS) Cells

Fibroblasts

Oct3/4  Sox2
retroviruses

Klf4  c-Myc

iPS Cells

Mouse iPS cells reported in 2006
Human iPS cells reported in 2007

2012 Nobel Prize in medicine awarded to Sir John Gurdon, Shinya Yamanaka
Specific transcription factors can directly induce cardiac myocyte differentiation from fibroblasts

In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes.
Summary:

1. Priming with Cell-Kro promotes cell adhesion, proliferation, survival, and migration after CSC transplantation to hearts with infarction. CSC derivatives remain for at least 1 month.

2. The priming method we developed is feasible for clinical application (30 min priming on ice prior to transplantation). One component of Cell-Kro is FDA-approved (Insulin).

3. Due to the prevalence of CTGF- and Insulin-based signaling throughout the body, it may be possible to graft many different cell types using CTGF-D4/Insulin.

4. Reprogramming technology may facilitate cardiac myocyte replacement.

5. Sub-epicardial niche may provide a delivery system to send cells and “payload” directly to infarcted cardiac tissue.
Special Thanks to:

Yoshitaka Iso, M.D., Ph.D.
Charla N. Poole, B.S.
Krithika S. Rao, M.S.
Sasha Aronshtam, Ph.D.
Ingrid Curril, Ph.D.
Calvin Yang, M.S.
Burton Sobel, M.D.
Sobel Lab members

Piero Anversa, M.D. (Harvard University)
Jan Kajstura, Ph.D. (Harvard University)

The National Institutes of Health