Cardiac Repair and iPS Cells

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Department of Medicine

Outline

- Background on cardiac cell therapy and iPSCs
- iPSC differentiation for relevant therapeutic cell products
- Preclinical animal cardiac studies with iPSCs and derivatives
- Safety issues
- Future directions

Disclosure:
Goals for Cell Therapy

Isolate well-characterized donor cell population

Develop matrices and grafts for tissue engineering

Test functional properties of cells or tissues in vitro

Transplantation into animal disease models

Strategies to prevent immune rejection

Cell or tissue delivery techniques

Efficacy
• Engraftment
• Integration
• In vivo function
• Disease regression/cure

Safety
• Absence of infectious agents
• No tumor formation
• Avoid cell-specific adverse effects, e.g. arrhythmias

Human Trials

Cardiac Repair with Cell Therapy

Many of the prevalent forms of heart disease are associated with cardiomyocyte loss via necrosis or apoptosis.

- Ischemic heart disease / MI
- Cardiomyopathies
- Certain arrhythmias
- Valvular heart disease

Many types of congenital heart disease are due to failure to develop specific cell lineages or form normal structures.
Cell Sources for Cardiac Repair

- Comparing cell sources
  - Unipotent, multipotent, pluripotent
  - Accessibility
  - Scalability
  - Autologous vs. allogeneic
  - Genetically modified
  - Commercial relevance
  - Ethics / Public policy

Modified from Dimmeler et al., 2005 JCI 115:572

Discovery Timeline: Reprogramming and Stem Cells

- 1962: Hematopoietic Stem Cells Proven
- 1963: Cloning tadpole nuclear transfer
- 1981: Rhesus ESCs
- 1995: Dolly sheep by nuclear transfer
- 1997: Mouse iPSCs
- 2006: Mouse ESCs
- 2007: Human ESC
- 2010: Human iPSCs

1st ESC Clinical Trial: Spinal Cord Injury
Thomson, Yamanaka
Geron
Human Pluripotent Stem Cells

**ES/g3CELLS/g3**

Skin samples are obtained and cells are grown

Four genes are introduced into the somatic cells

After four weeks iPS cells can be seen and cultured

**iPS CELLS**

**ES CELLS**

Embryo undergoes multiple divisions

Blastocyst is formed, and inner cell mass is extracted

Stem cell lines are created as extracted cells grow and divide

- NEURONAL CELLS
- HEMATOPOIETIC CELLS
- CARDIOMYOCYTES
- RENAL CELLS
- HEPATOCYTES

**NEURONAL CELLS**

**HEMATOPOIETIC CELLS**

**CARDIOMYOCYTES**

**RENNAL CELLS**

**HEPATOCYTES**

**iPSC Technology Rapidly Evolving**

- **Early Generation**
  - Retroviral/lentiviral
  - Plasmid-mediated
  - Minicircle vectors
  - Transposon / transposase

- **Footprint-free methods**
  - oriP/EBNA – episomal vectors
  - Sendai virus
  - Adenovirus
  - Protein-mediated
  - Synthetic mRNA
  - miRNAs
  - Small molecules
  - Combinations
Qualification of iPSC Line

- Culture characteristics of iPSCs – proliferative, colonies of tightly packed cells (like ESCs)
- Expression of pluripotency-related proteins
  - SSEA3, SSEA4, Tra-1-60, Oct4, Nanog
- Pluripotency gene expression pattern
  - POUF5, NANOG, LIN28, TERT, DPPA4, PODXL,…
- Karyotypically normal
- Absence of transgenes used for reprogramming
- Absence of infectious diseases, e.g. HIV, HBC
- Differentiation capacity of cells to three primary germ layers
  - in vitro – embryoid bodies, in vivo - teratomas
- Identity testing – DNA fingerprinting (STR analysis)

Cardiac Applications of iPSCs

- Basic biomedical research using human heart cells
  - Cardiac development
  - Cardiac cell biology, physiology, etc.
- Patient- and disease-specific iPSC cells for modeling inherited cardiac diseases
- Drug discovery and development including safety testing
- Potential therapeutic applications
Human iPSCs – Potent Possibilities for Cell Therapies

- Undifferentiated cells
  - Banks
- Differentiated cell lineages
  - Endothelial cells
  - Vascular smooth muscle cells
  - Cardiomyocytes
  - Mesenchymal stem cells
  - Other
- Mixed differentiated preparations
- Combinations of cell lineages
- Genetically modified

Needed - Differentiation Protocols

- Defined, xenofree reagents
- Reproducible
- Robust – work for multiple iPSC lines
- Cost efficient, e.g. peptide GFs $$
- Scalable
- Output key properties
  - Yield per input iPSC
  - Purity of desired cell lineage
  - Cell lineage critical characteristics
  - Lack undifferentiated cells
Cardiomyocyte Differentiation Challenge

- mESC research developed embryoid body (EB)-based differentiation using qualified serum
  - mESCs culture conditions different from hESCs
- Cardiac differentiation from hPSCs:
  - Inefficient with many methods
  - Poorly reproducible
  - Cell line-dependent

From hPSC to Cardiomyocyte

<table>
<thead>
<tr>
<th>CM Lineage</th>
<th>Transcription Factors</th>
<th>Cell-Surface Markers</th>
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<tbody>
<tr>
<td>ESC / iPSC</td>
<td>Oct4, Nanog, Sox2</td>
<td>Tra-160, SSEA4, EpCAM</td>
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<td>Epithelial-Mesenchymal Transition</td>
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<td>Mesoderm Progenitor</td>
<td>Oct4</td>
<td>NCAM, SSEA1</td>
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<td>BMP4, Activin A, FGF-2 Wnt3a, Insulin</td>
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<td>T, MIXL1</td>
<td>KDR, PDGFRα</td>
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<tr>
<td>BMP4, Activin A, FGF-2 Wnt3a</td>
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<tr>
<td>Cardiac Mesoderm</td>
<td>Mesp1</td>
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<tr>
<td>Wnt, Dkk1</td>
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<tr>
<td>Heart Field Specific Progenitor</td>
<td>Nkx2.5, GATA4 (TBX5/Is1/TBX20)</td>
<td>SIRPA</td>
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<tr>
<td>Embryonic CM</td>
<td>Nkx2.5, GATA4</td>
<td>SIRPA, VCAM-1</td>
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Cardiac Differentiation Strategies

Manipulating Extracellular Matrix for Optimizing Cardiac Differentiation

Functions as adhesive substrate

Provides structures for developing tissues and organs

Presents growth factors to their receptors

Sequesters and stores growth factors

Senses and transduces mechanical signals


Modified from Rozario and DeSimone, 2010 Devel Biol 341:126
Matrix Sandwich Method

Matrix Sandwich: Other Cell Types

Zhang et al., 2012, Circ Res 111:1125
Biphasic Modulation of Wnt Signaling for Directed Cardiac Differentiation

Small Molecule Wnt Signaling Modulation Produces Robust Cardiogenesis

iPS-CMs from Multiple hPSC Lines

<table>
<thead>
<tr>
<th></th>
<th>α-actinin/Nuclei</th>
<th>MLC2a/Nuclei</th>
<th>Merge</th>
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</table>

EP Shows Multiple Types of iPS-CMs

- Nodal-like (~5%)
- Atrial-like (~15%)
- Ventricular-like (~80%)

Gisela Wilson
Human iPS-Engineered Cardiac Tissue:
3D Model of Functional Human Myocardium

• Enzymatically isolated iPSC-CMs
• Mix with Fibrin Gel

Kunil Raval
J. Carter Ralphe
Toy De Lange

Cardiac Differentiation Summary

- Differentiation protocols for CMs have improved markedly from EB- and serum-based.
- Monolayer cultured hPSCs protocols can provide reproducible and robust generation of CMs.
  - Matrix-promoted with sequential application of GFs enables effective directed differentiation of CMs.
  - Biphasic modulation of Wnt signaling can promote efficient cardiogenesis using only small molecules.
- Cardiac tissue engineering is possible
- Caveats
  - Quality, undifferentiated input hPSCs critical
  - Medium for hPSC maintenance can impact outcome
  - Line to line variability in effectiveness of protocol
  - Fully defined, xenofree not fully achieved for some
Outline

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Disclosure:

**miPSCs Repair Immunocompetent Mouse Post-MI – Multilineage**

miPSCs and Tumor in Immunodeficient Mouse Post-MI

Subsequent studies in mice and rats raise questions of tumorigenesis of miPSCs (Ahmed et al., 2011, Regen Med 6:171; Zhang et al., 2011, PlosOne 6:e19012).

Mouse siPSCs and Post-MI Repair

- Reprogram skeletal myoblasts with DNMT inhibitor only to iPSC
- Differentiate in EBs and isolate contracting regions

Pasha et al., 2011 PLoS ONE 6(8): e23667
miPSC-CMs+miPSC-ECs+MEFs in Peritoneal Patch

- Combined on peritoneal patch
  - NCX-selected iPSC-CMs
  - CD31 selected iPSC-ECs
  - MEFs
- Transplant cellularized patch 7 d post-MI

Dai et al., 2011 JACC 58:2118

hiPSC-ECs and SMCs in Fibrin Gel to Pig Ischemia/Reperfusion Model

- Clinically relevant I/R porcine model
- hiPSCs
  - ECs
  - SMCs
- Cells delivered via epicardial fibrin patch
- Detailed cardiac MRI evaluation

Xiong Q et al. Circulation 2013;127:997-1008
In vivo Myocardial ATP Turnover Rate Improved in BZ from $^{31}$P MRS

Xiong Q et al. Circulation 2013;127:997-1008

Engraftment and Vascularization by hiPSC-ECs and SMCs in Pig

Xiong Q et al. Circulation 2013;127:997-1008
iPSC and Preclinical Animal Post-MI Studies Summary

- Initial studies using undifferentiated miPSCs in post-MI mouse heart showed promising effects on cardiac structure and function, but questions arise regarding teratomas.
- Studies using differentiated derivatives of miPSCs have shown encouraging results including the application of tissue engineering with patches of contracting cells.
- Translation to large animals with clinically relevant models has just begun with studies of hiPSC derivatives (EC and SMC) in porcine post-MI model with improvements in function, structure, metabolism and vascularization.
- Just the beginning of such animal research....

Safety: Tumorigenicity

- Issues related to reprogramming
  - c-myc transgene used
  - Integration mutagenesis by transgenes
  - Acquired mutations, e.g. oncogenes
  - Aberrant epigenetic status
  - Lineage of origin
- Testing of cellular products
  - Tumor in tissue/disease of interest?
  - Teratoma/tumor formation in NOD/SCID?
  - Standards not yet available
Safety: Immunogenicity?

- Animal products in culture or processing?
- Undifferentiated miPSCs form teratomas that are immune rejected in syngeneic mouse model in contrast to mESCs (Zhao et al, 2011, nature 474:212)
  - Attributed to aberrant protein expression
    - Hormad1, Spt1
  - Another group has not observed in differentiated NPCs (Okano et al., 2013 Circ Res 112:523)
- Testing of human cellular products?
  - Unknown – humanized mouse models?

Future Challenges

- Optimize and standardize methodology for generation of clinical grade iPSC lines
  - Defined, xeno-free reagents; footprint-free, optimal cell source
  - Efficient, minimize genomic abnormalities, fully reprogrammed epigenetic status, robust differentiation capacity
- Advanced qualification of iPSCs
  - Copy number variations – CGH arrays?
  - Somatic mutations – whole genome sequencing?
  - Epigenetic status – DNA methylome?
- Accessible sources for uniform iPSC and iPSC derivatives as CMs
  - Banking, HLA matching
- Improve differentiation protocols to reduce heterogeneity and improve reproducibility
- Preclinical large animal cardiac models for hiPSC products?
- Defining safety
  - Tumorigenicity assays?
  - Immunogenicity assays?
  - Cardiac-specific safety, e.g. arrhythmias
Balancing Benefits vs Risks of iPSCs

- Autologous
- Unlimited quantities
- Multiple cell lineages
- Not senescent

- Tumor formation
- Immunogenicity
- Epigenetic memory
- Somatic mutations
- Copy number variation
- Genetic disease risk