Cardiac stem cells
development, disease and repair

Leiden University Medical Center
Anatomy and Embryology

Robert Passier
r.passier@lumc.nl
What kind of stem cells can be identified?

• **adult or somatic stem cells**
  – Present in all tissues and organs (adult or fetal) with the capacity to repair after injury
  – Differentiation capacity (uni- or multipotent) and number of cells are limited
  – Not ethically sensitive since autologous cells (from the patient) can be transplanted.
  – Endogenous activation/differentiation may be possible

• **embryonic stem cells**
  – Derived from blastocyst-stage embryo’s (in human:1998)
  – Pluripotent
  – Ethically sensitive, but many cell(s) lines available and can differentiate to all cells of the human body

• **Induced pluripotent stem cells**
  – Derived from reprogrammed somatic cells (in human:2007)
  – Pluripotent (similar to ESCs)
  – Not ethically sensitive, since cells can be derived from adults (patients). Could be used for autologous transplantation
Stem cells for cardiac repair

- Loss of cardiomyocytes in cardiovascular diseases (myocardial infarction)
- Intrinsic myocardial regeneration is limited
Bone marrow cells and myocardial regeneration. a, Myocardial infarct (MI) injected with Lin- c-kitPOS cells from bone marrow (arrows). Arrowheads indicate regenerating myocardium; VM, viable myocardium.

Adult stem cells for cardiac repair

- Clinical trials with bone marrow cells with limited success
  - Slight improvement of heart function
  - No cardiac regeneration

- Mesenchymal stem cells (MSCs)
  - Stromal cells obtained from the bone marrow, but also from many other tissues
    - Adipose tissue, umbilical cord blood, placenta, pericvascular tissues, etc.
  - Self-renewal capacity
  - Multipotent differentiation capacity
    - Chondrocytes, osteoblasts, adipocytes, cardiomyocytes

- Role of MSCs in cardiac repair
  - Improved heart function
    - Migration to injury site
    - Immunosuppressive properties
    - Increased vascularization
    - Release of growth factors (VEGF, IGF-1)
    - Cardiac differentiation from MSCs is limited
Clusters of primitive and early committed cells could be found in the heart

Small cluster of c-kit+ cells (green) positive for Nkx2.5 (white)

Lin− c-kit+ CSCs injected into an ischemic heart resulted in the formation of blood-carrying new vessels and myocytes

Markers identifying cardiac stem cells:
- C-kit+
- Sca-1+
- Isl-1+
- Flk-1+
- SP+

Cardiospheres of culture explants of human heart biopsies represent a potential source of endogenous cardiac stem cells
- Clinical trial in MI patients (CADUCEUS)

Epicardial cells (WT1+/TBX18+) represent another cell population that may contribute to endogenous repair under the right conditions (Smart et al Nature 2011)
- Enhanced regeneration in the presence of Thymosin Beta 4
Human Pluripotent Stem Cells

generation of cardiac progenitor cells and cardiomyocytes
embryonic stem cells
Culturing human embryonic stem cells

hESC on mouse feeders, ready to passage

hESC, 1 day after transfer

cutting colonies
hESC-END-2 co-culture
improving cardiomyocyte differentiation

undifferentiated hESC → END-2 → beating area

12 days

cardiomyocyte differentiation efficiency

Mummery et al. 2003
2003
20% FCS

Passier et al. 2005
2005
no FCS
24-fold ↑

Freund et al. 2008
2008
no FCS
no insulin
3-fold ↑

72-fold ↑
hESC differentiating to cardiomyocytes

Differentiating hESC-CM follow “waves” of expression comparable to in vivo cardiac development!

Beqqali et Stem Cells 2006
Cardiac proteins in hESC, human fetal and adult cardiomyocytes (CM)
Electrophysiological characterization of hESC-CM

On average >85% of cardiomyocytes show a ventricular-like action potential.
Human pluripotent stem cell-derived cardiomyocytes cell transplantation for cardiac repair?
Effect on cardiac function?

Model of acute myocardial infarction
Male SCID mice (n=13-15 per group)
- MI (LAD ligation) +
  - 1 million GFP-HES3 from beating areas END-2 co-culture (20% CM)
  - 1 million non-CM differentiated from GFP-HES3
- MRI (9.4 T) after 2 days, 4 weeks, 12 weeks

Cardiac function improvement at 4 weeks not sustained!
Why do we need human stem cell-derived cardiomyocytes?

hPSC-derived cardiomyocytes may be used as:

- a possible source for cell replacement therapy
- an alternative for heart transplantation in patients with end-stage heart failure (van Laake et al. Stem Cell Research 2007)
- a human in vitro model for:
  - cardiomyocyte differentiation or cardiac development (Beqqali et al.
  - drug toxicity (Braam et al. Stem Cell Research 2010)
  - cardiac disease
- target and drug discovery

Higher predictability and success can be achieved with more homogeneous cardiac subtype populations or controlled mixtures of cells.
Identification of cardiac subtype populations

Reporter lines for cardiac conduction system
Building a cardiac reporter line

Nkx genomic locus

Nkx targeting construct

hESC-Nkx-GFP line

epiplast

hESCs

gastrulation

ectoderm

mesoderm

endoderm

CPCs

CM

differentiation

UH 1d 3d 6d 9d 12d
EGFP expression by the endogenous NKX2-5 promoter

Homologous recombination following electroporation in hESC

Beating differentiating hESC
Controlled differentiation

Elliott et al. Nat Methods 2011
cardiac cell differentiation and purification

Defined, HT differentiation

Differentiation in 96-well plates (scalable technology)

> 50% Cardiomyocytes

Purify cardiomyocytes: sorting (SIRP + VCAM1)

Elliot et al. Nat. Meth. 2011, 8:1037
Generation of a cardiac mesodermal hESC line

Generated in Nkx2.5-GFP background: double transgenic hESC line (Mesp1-mCherry/Nkx2.5-GFP)

MESP1 reporter hESC line

- Mesp1 genomic locus
- Mesp1 targeting construct
- hESC-Mesp1-mcherry line

Cardiac differentiation

UH 1d 3d 7d 10d

- d1-d3
- BMP4
- Activin-A
- Chir (GSK-inhibitor)

- Xav (d3-d7)
- NKX2-5-eGFP
Mesp1-mCherry/Nkx2.5-GFP reporter hESC-line

**RT-PCR**

![Graphs showing relative quantity of mCherry and Mesp1 over time from D1 to D12.]

**FACS**

![FACS analysis plots for mCherry expression at different time points (D1, D3, D4, D8, D12).]
Mesp1-mCherry expressing Cells at Day 3 of Differentiation
How do we get cardiac subtype populations? (atrial, ventricular, pacemaker cells)
Heart development at a glance

Stage:
- Cardiac crescent
  - Mouse embryo day: E7.5
  - Human embryo day: Day 15
- Linear heart tube
  - Mouse embryo day: E8
  - Human embryo day: Day 20
- Looping heart
  - Mouse embryo day: E9
  - Human embryo day: Day 28
- Chamber formation
  - Mouse embryo day: E10
  - Human embryo day: Day 32

Key:
- WNT
- BMP
- ACTIVIN/NODAL
- SHH
- NOTCH
- RA
- NODAL
- LEFTY
- NRG
- RA
- NOTCH

- MESP1/2
- NKX2.5
- GATA4
- TBX5
- MEF2
- SRF
- PITX2
- HAND1
- HAND2
- HEY1
- HEY2
- IRX4
Retinoic acid in heart development

Gassanov et al. Differentiation 2008: RA induces atrial differentiation in mES

Keegan et al. Science 2005
Expression of RALDH2 in differentiating hESC-CM

![Graph showing relative expression of RALDH2 over time points D2 to D14.]

**Time point of differentiation**

- D2
- D5
- D8
- D11
- D14

Relative expression

**VEGF, BMP4, ACT-A**

- D0: Spin aggregation of hESC
- D3: Refreshing GF’s
- RA: Plating
- D7: Analysis
- D13: DAPI

**Image:** RALDH2 expression with DAPI stain.
Retinoic acid treatment: shift from ventricular to atrial cells

Beating frequency

- HAND1
- HEY2
- IRX4
- MLC2V

ventricular genes

atrial genes
ION CURRENTS CONTRIBUTING TO ATRIAL & VENTRICULAR ACTION POTENTIALS

Supported by Zhang et al. Cell Research 2011: RA signalling affects differentiation of atrial and ventricular cells
Action potential properties of RA treated cardiomyocytes

A

B

C

D
Conclusions

• Mesenchymal stem cells and cardiac stem cells are promising cell sources for the treatment of cardiac disease.
  – Transplantation, tissue engineering, endogenous activation

• Human pluripotent stem cells for transplantation: tissue engineering using mixtures of cardiac cells from defined differentiation cultures will be the next step

• Genetic cardiac reporter lines faithfully recapitulate the “in vivo” lineage
  – Molecular mechanisms for expansion and differentiation can be studied

• Refined protocols enable cardiac subtype specification (retinonic acid → atrial CMs)
  – Advantageous for tissue engineering, drug screening, disease modeling
Acknowledgements

Harsha Deepti Devalla
Marcelo Ribeiro
Sabine Den Hartogh
Verena Rönz
Jantine Monshouwer
Chantal Schreurs
Marie-Christine Weller
Yann Decker
Juan Antonio Guadix

David Elliot
Andrew Elefanty
Ed Stanley

Christine Mummery
Richard Davis
Dorien Ward

Arie Verkerk