Dedifferentiation of Adult Cardiac Myocytes

Joanne Stocks

Icahn School of Medicine at Mount Sinai
Background

- Adult mammalian myocardium has no regenerative capacity.

- Cardiomyocyte loss as a result of injury or disease is irreversible.

- Regeneration of the myocardium is a new area being investigated for future therapy against heart failure.
Current Therapeutic Ideas

- Cell replacement strategies - potential to restore cardiac function?
- Promising results from both basic animal studies and clinical trials using:
  - embryonic stem cells
  - skeletal myoblasts
  - haematopoietic stem cells
  - endothelial progenitor cells
  - mesenchymal stem cells
Current Therapeutic Ideas

- Reverse terminal differentiation
  - Activating committed progenitor cells
  - Encourage differentiated cells to re enter cell cycle

- Could cardiac derived stem/progenitor cells differentiate into cardiac myocytes?

- Could grafted healthy myocytes couple with host myocytes in a diseased heart?
- Myocyte proliferation reported in vivo but not in vitro.

- Stem cell differentiation?

- Cardiac Environment?

- Paracrine factors released by stem cells?
“Paracrine factors released by modified MSCs potentially can exert effects on various aspects of cardiac pathophysiology such as myocardial cell survival, angiogenesis, remodelling, contractility, and even myocyte regeneration. “

Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. M Gncechi, VJ Dzau et al. Nature Medicine 11, 367 - 368 (2005)
Hypothesis

Paracrine factors released by C kit positive cardiac derived progenitor cells can have a protective effect on adult cardiac myocytes.

Paracrine factors released by C kit positive cardiac derived progenitor cells induce dedifferentiation of adult cardiac myocytes.
Method

- C kit$^{+ve}$ cardiac derived progenitor cells (CDPC) and cardiomyocytes were isolated from 6-8 week old adult rats (200-250g).
- C kit$^{+ve}$ CDPC’s grown in culture for 4 days.
- Supernatant is collected and diluted 50:50 with αMEM, 10% FCS and AraC.
- Myocytes allowed to adhere for 2-12hrs on laminin coated plates before culturing with conditioned media.
Changes in Myocyte Morphology in Culture

Day 0 40x

Day 1 40x

Day 2 40x

Day 3 40x
Changes in Myocyte Morphology in Culture

Day 4 40x
Day 5 40x
Day 6 40x
Day 7 40x
Single cell beating rate per minute
Expression of Connexin 43 mRNA

Normal Adult Myocyte Media

Conditioned Stem Cell Media

Day 3  Day 4  Day 5
Expression of bMHC mRNA

Normal Adult Myocyte Media
Conditioned Stem Cell Media
Expression of ANF mRNA

Normal Adult Myocyte Media

Conditioned Stem Cell Media
Our cells express neonatal phenotype

Exciting considering previous work in

- Cardiomyocytes derived from mouse embryonic stem cells.
- Primary cultures of foetal cardiomyocytes transplanted into the connective tissue of mice or dogs.
- Cardiomyocyte transplantation from rat neonatal myocytes.
Improved clinical outcomes observed using these myocytes

- Associated with smaller infarcts,
- Prevented cardiac dilatation
- Prevented remodelling
- Improved myocardial performance.
If adult myocytes with neonatal phenotype are to be used to repair the heart they need to be collected from culture once dedifferentiated and to be remain viable once they have been cryopreserved

- **Cells cultured for 7 days in conditioned media**

- **Frozen for 72hrs (90% FCS 10% DMSO)**

- **Re-plated and filmed 6hrs later**
“From a therapeutic standpoint, intrinsic cardiomyocyte proliferation rates as low as 0.05% to 0.1% could, over the course of many months, result in a significant increase in cardiomyocyte number and, consequently, partial restoration of myocardial function in a diseased heart.”

C Kit and receptor
Nanog
Isl-1

Stem cell associated markers
Cyclin D1 + CDK4

CyclinD-CDK4

E2F, DP1, RB

CDK2 + Cyclin E

CyclinE-CDK2

p53

Cdkn1a (p21)

G1 → S

CDK2 + Cyclin A

CyclinA-CDK2

G2 → M

Cdkn2a+2b (p15+p16)

p53

G1 → S

G2 → M
Day 0  Day 4  Day 14

Cyclin D1, D2
Cyclin E

p21, p16, p15
Rb  p53
Cyclin D1 + CDK4 → CyclinD-CDK4

Cyclin A + CDK2 → CyclinA-CDK2

Cyclin E + CDK2 → CyclinE-CDK2

E2F → DP1

RB → E2F

Cdkn2a+2b (p15+p16) → X

Cdkn1a (p21) → G1 → S

Cyclin A-CDK2 → G2 → M

Cyclin B-CDC2 → Initiates G2 to M

Cyclin B → CDC2

Initiates G2 to M
4 day untreated cells
Untreated 4 dys
Conclusion

- Secreted factors from cardiac progenitor cells appear to rapidly dedifferentiate adult myocytes to a neonatal phenotype and morphology.
- Secreted factors from cardiac progenitor cells may have a protective paracrine effect on adult myocytes.
- Dedifferentiated adult myocytes can be cryopreserved and regain contractile properties.
- **Dededifferentiated adult myocytes have a greater potential to proliferate.**
Future

- Can adult dedifferentiated cells integrate into host myocardium?
- Can this conditioned media cause dedifferentiation in vivo?
- Can injecting conditioned media encourage proliferation to repair the injured myocardium?
- Can paracrine factors from cardiac progenitor cells have a protective effect on injured myocardium?