Induced Pluripotent Stem Cells and Direct Cardiac Reprogramming

Solving Barriers for a Powerful Future: The 2016 New Experimental and Clinical Information

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Adult Hearts Have Little Regenerative Capacity

Normal heart
Fibroblasts CMs

(Ieda et al., Dev Cell, 2009)

Normal heart
Cardiomyocytes

Myocardial infarction (MI)
Fibroblasts
1. Transplantation of induced pluripotent stem cells (iPSCs)-derived cardiomyocytes

   - Fibroblasts
   - iPSCs
   - Cardiomyocytes

   

   OCT4, SOX2
   KLF4, c-MYC


2. Direct reprogramming of resident cardiac fibroblasts into cardiomyocytes (direct cardiac reprogramming)

   - Cardiac fibroblasts
   - Cardiomyocytes

   Gata4, Mef2c, Tbx5

Combination of Stem Cell-specific Factors Convert Fibroblasts to iPSCs

Fibroblasts $\rightarrow$ $OCT4$, $SOX2$, $KLF4$, $c$-MYC $\rightarrow$ induced pluripotent stem cells (iPSCs)


- myocytes
- iPSCs
- Neuron
- Blood
- Liver
Generation of CMs through iPSCs

- **Generation of iPSC**
  - Stems and Specific Factors
  - Oct3/4, Sox2, Klf4, c-Myc

- **Differentiation**
  - iPSC
  - Cardiomyocyte
  - Neuron
  - Blood
  - Hepatocyte

- **Purification**
  - FACS

- **Complete**
  - Cardiomyocyte
Regeneration using iPSC-CMs

iPSC-CM transplantation
1. Complicated/long process
2. Tumor by iPSC contamination
3. Poor survival of the cells
Purification of CMs with Culture Conditions

CMs can use lactate for ATP synthesis

- **CMs**
  - Mitochondria rich
  - Use lactate for ATP synthesis

- **iPSCs**
  - Mitochondria poor
  - Use only glucose for ATP synthesis

Glucose-/lactate+ culture media may purify CMs and kill iPSCs

CMs survived under glucose-/lactate+

Purification of CMs > 90%

(Tohyama et al., Cell stem Cell, 2013)
Monkey iPSC-CMs Regenerate MI Hearts

Autologus iPSCs  →  Allogenic iPSCs (iPSC bank)

Allogenic monkey iPSC-CMs survived in MI hearts with immunosuppresants.

iPSC-CMs Improved EF

iPSC-CMs induced VT

(Shiba et al., Nature, 2016)
Strategies for Cardiac Regeneration

1. Transplantation of induced pluripotent stem cells (iPSCs)-derived cardiomyocytes

   1.5 OCT4, SOX2
   1.25 KLF4, c-MYC

   Fibroblasts → iPSCs → Cardiomyocytes


2. Direct reprogramming of resident cardiac fibroblasts into cardiomyocytes (direct cardiac reprogramming)

   Gata4, Mef2c, Tbx5

   Cardiac fibroblasts → Cardiomyocytes

Direct Reprogramming

Induced CMs (iCMs)

Lactate

Generation of Cardiomyocytes from Fibroblasts

Original Method

Fibroblast

Generation of iPSC

Stem Cell-Specific Factors

Oct3/4 Sox2 Klf4 c-Myc

iPSC

Differentiation

iPSC

Cardio myocyte

Neuron

Blood

Hepatocyte

Purification

FACS Lactate

Cardio myocyte

Complete
Direct cardiac reprogramming

Induced CMs (iCMs)

Fibroblast

Original Method

Generation of iPSC

Differentiation

Purification

Complete

Stem Cell-Specific Factors

Oct3/4, Sox2, Klf4, c-Myc

iPSC

Cardiac myocyte

Neuron

Blood

Hepatocyte

FACS

Lactate

Cardiac myocyte

Fibroblast

Novel Method

Generation of iCM

Direct cardiac reprogramming

Induced CMs (iCMs)
Regeneration using iPSC-CMs

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Regeneration by Cardiac Reprogramming

**iPSC-CM transplantation**
1. Complicated/long process
2. Tumor by iPSC contamination
3. Poor survival of the cells

**Cardiac reprogramming**
1. Simple/fast process
2. No risk of tumor formation
3. No need of transplantation
Discovery and Progress of Cardiac Reprogramming

1. Cardiac reprogramming in mouse and human cells

   Mouse fibroblasts → Gata4/Mef2c/Tbx5 (GMT) → Induced cardiomyocytes (iCMs)


2. Heart regeneration by in vivo cardiac reprogramming

   Mouse MI → Cardiac fibroblasts → GMT → iCMs

   (Inagawa et al., Circ Res, 2012)
Gata4/Mef2c/Tbx5 Are Cardiac Reprogramming Factors in Mouse

αMHC-GFP TG mouse

Screening for cardiac reprogramming factors

GMT converted fibroblasts into iCMs
Gata4/Mef2c/Tbx5 Are Cardiac Reprogramming Factors

(leda et al., Cell, 2010)
Addition of Cytokines Improved Cardiac Reprogramming Efficiency

(Yamakawa et al., Stem Cell Reports, 2015)
How about in human?

Human fibroblasts \[\xrightarrow{\text{Gata4/Mef2c/Tbx5/} + ?} \] Human iCMs
Gata4/Mef2c/Tbx5/Myocd/Mesp1 Are Human Cardiac Reprogramming Factors

Human fibroblasts

Gata4/Mef2c/Tbx5/Myocd/Mesp1 (GMTMM)

Human iCMs

Gata4/Mef2c/Tbx5/Myocd/Mesp1

(Wada et al., PNAS, 2013)
(Muraoka et al., EMBO J, 2014)
Human iCMs Beat Synchronously in co-culture with other CMs

GFP-tagged human iCMs

(Wada et al., PNAS 2013)
In Vivo Cardiac Reprogramming by GMT

Mouse MI model

Gata4/Mef2c/Tbx5 (GMT)

Retrovirus GFP infected fibroblasts

GFP

Actinin

Merged

(Inagawa et al Circ Res, 2012)
In Vivo Reprogramming Improved Cardiac Function

Gata4/Mef2c/Tbx5 reduced fibrosis

Gata4/Mef2c/Tbx5 improved cardiac function

Echocardiogram

Control

Gata4/Mef2c/Tbx5
1. Cardiac reprogramming in mouse and human cells

Mouse fibroblasts $\xrightarrow{\text{Gata4/Mef2c/Tbx5}}$ Induced cardiomyocytes (iCMs) 


2. Heart regeneration by in vivo cardiac reprogramming

Mouse MI $\xrightarrow{\text{GHz}}$ iCMs 

(Inagawa et al., Circ Res, 2012)
Regeneration by Cardiac Reprogramming

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