Trans-differentiation of Human Fibroblasts to Endothelial Cells: Role of Innate Immunity


Houston, TX; Stanford, CA; Cincinnati, OH

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Shinya Yamanaka’s Discovery:
- 2012 - Nobel Prize for Medicine
- Forced reprogramming of somatic cells to induced pluripotent stem cells (iPSCs)
  - By expression of the TFs: Oct4, Sox2, Klf4 and cMyc ("OSKM")

Disadvantage:
- Cells are transfected by viral vectors
- Induce overexpression of TFs → cause global changes in expression and activity of epigenetic modifiers.
- Only small percentage of cells transform (≈2–4 weeks)
Introduction

Adapted from Meylan et al., 2006
Aim

- **Ideal for Clinical Application:** Transdifferentiation of cells, but avoid genetic manipulation
- Generation of safe and functional induced ECs ("iECs") from fibroblasts.
- Use small molecule to activate TLR3 and inductive growth cues, to induce trans-differentiation.
Polyinosinic-polycytidylic acid

- Poly(I:C) a synthetic analog of double stranded RNA (dsRNA)
- A molecular pattern associated with viral infection
- Poly(I:C) is recognized by Toll-like receptor 3 (TLR3)
- Activates TFs interferon regulatory factor 3 (IRF3), NF-κB and AP-14
- Triggers production of inflammatory cytokines and chemokines such as TNF-α, IL-6 and CXCL10
Timeline

Direct reprogramming of fibroblasts to induced endothelial cells (iECs)

- BJ human new-born foreskin fibroblast cells

Figure 1A
Detection of EC-Specific Markers After 28 Days of Differentiation

Human foreskin fibroblasts (BJ)
Expansion of Endothelial Cell Markers

Human foreskin fibroblasts
Expression of Endothelial Cell Markers

Human foreskin fibroblasts

Figure 1D; S1C-F
Expression of Endothelial Cell Markers

Human foreskin fibroblasts

CD31/DAPI

CD144/DAPI

vWF/DAPI

Fibroblasts

iECs

Fibroblasts

iECs

Fibroblasts

iECs

50 μm
Uptake of Acetylated LDL & Tubular Network Formation

Human foreskin fibroblasts

Ac-LDL/DAPI

Network formation
Nitric Oxide Production by iECs

Human foreskin fibroblasts
Expression of Angiogenic Cytokines

Human foreskin fibroblasts
Capacity to Form Capillaries

*InVivo*

Human foreskin fibroblasts

Figure 1J; S1K,L
Sorting of GFP positive from GFP negative cells

Tie2GFP mice tail-tip fibroblasts
Detection of EC-Specific Markers After 28 Days of Differentiation

Tie2GFP mice tail-tip fibroblasts
Uptake of Acetylated LDL & Tubular Network Formation

Tie2GFP mice tail-tip fibroblasts

In vitro

In vivo

Figure 2 E,F,G
Arterial EC and Mesodermal Marker Expression Increased

Tie2GFP mice tail-tip fibroblasts

Arterial specific markers

Mesoderm specific markers

Figure S2A,B
Histone modifications during direct reprogramming to iECs

Tie2GFP mice tail-tip fibroblasts

H3K4me3

H3K27me3

Figure S3A,B
Transcriptional profiling of induced-ECs

Tie2GFP mice tail-tip fibroblasts
Therapeutic potential of iECs in a model of peripheral arterial disease
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Tie2GFP mice tail-tip fibroblasts
Therapeutic potential of iECs in a model of peripheral arterial disease

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Therapeutic potential of iECs in a model of peripheral arterial disease

Tie2GFP mice tail-tip fibroblasts

[Images of mouse hindlimbs showing Day 18, Control, iECs, and HMVEC conditions]
Innate immunity enables efficient transdifferentiation of fibroblasts to iECs

TLR3 knock-down mice tail-tip fibroblasts
Reduced Capacity to Incorporate Ac-LDL

TLR3 knock-down mice tail-tip fibroblasts

Ac-LDL/DAPI

Scram - iECs
TLR3 KD - iECs

Ac-LDL Fluorescence Intensity (Compared to Scramble)

Acetylated-LDL fluorescent intensity
Failure to Form Capillary-like Networks

TLR3 knock-down mice tail-tip fibroblasts
Changed Gene & Protein Expression

TLR3 knock-down mice tail-tip fibroblasts

**CD31**

**CD144**

**KDR**

**vWF**

**eNOS**

Figure S5A-E
Changed Gene & Protein Expression

TLR3 knock-down mice tail-tip fibroblasts

- **CD31/DAPI**
  - Fibroblasts
  - Scram-iECs
  - TLR3KD-iECs

- **CD144/DAPI**
  - Fibroblasts
  - Scram-iECs
  - TLR3KD-iECs

- **vWF/DAPI**
  - Fibroblasts
  - Scram-iECs
  - TLR3KD-iECs

Scale bar: 100µm
Heat Map of Genes Differential Expression in iECs

TLR3 knock-down mice tail-tip fibroblasts
Heat Map of Genes Differential Expression in iECs

TLR3 knock-down mice tail-tip fibroblasts

Angiogenesis essential genes activated in iECs:
- GJA4
- HOXA1
- HOXA3
- HOXB3
- HEY2
- GBX2
Reduced CD144+ Expression

TLR3 knock-out mice tail-tip fibroblasts

Figure S6
Summary

- TLR3 agonist and endothelial growth factors is sufficient to transdifferentiate human fibroblasts to iECs

- Similarity to human microvascular endothelial cells:
  - Morphologically, immunohistochemically and transcriptional profile

- Administration of iECs improved perfusion and reduced tissue injury in ischemic hindlimb

- Absence of Poly I:C and ECF did not differentiate the fibroblasts into iECs

- TLR3 or NFKB knockdown each reduced the generation of iECs using this protocol

Thank you for your attention!
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