A Chemical Approach to Controlling Cell Fate

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Therapeutic strategies toward regenerative medicine

Isolation of tissue specific cells
Differentiation
Reprogramming into iPSCs
IPSC differentiation (Brain, heart, pancreas, etc.)

The small molecule approach
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Some of past examples of synthetic small molecules controlling cell fate

- Pluripotin: Self-renewal of ESCs
- Neuropathiazol: Neuronal diff. of adult NSCs
- Purmorphamine: Osteogenesis of MSCs
- Pluripotin
- Reversine reprogramming
- Thiazovivin/Tzv
- Pyrintegrin/Ptn
- QS11

HTS identified novel survival promoting compounds

HTS measured cell viability:

- Delta H4
- Nanog
- SSEA4

Tzv's activity depends on functional E-cadherin in ECM-free condition

TUNEL positive cells (%)

Matrigel Non-coating

DMSO Tzv

Ptn Tzv + E-Cad Ab

Xu et al., PNAS 107, 8129-8134, 2010
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**Tzv stabilizes E-cadherin in ECM-free condition**

Tzv stabilizes E-cadherin in ECM-free condition. The graph shows the protein and mRNA levels of E-cadherin under different conditions.

**Tzv is a novel ROCK inhibitor and Rho-ROCK-myosin axis regulates cell-cell and cell-ECM interactions**

Tzv is a novel ROCK inhibitor and Rho-ROCK-myosin axis regulates cell-cell and cell-ECM interactions. The images illustrate the effects of various inhibitors on cell morphology.

**Cell-cell interaction and cell-ECM interaction regulate each other through Rho pathway and both contribute to survival of hESCs**

Cell-cell interaction and cell-ECM interaction regulate each other through Rho pathway and both contribute to survival of hESCs. The graph shows the activation levels of RhoA-GTP and E-cadherin under different conditions.
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### Adhesion mechanisms control stem cell survival and fate

- **Cell-cell interaction**
- **Cell-ECM interaction**
- **Anti-differentiation and pluripotency**
- **E-cadherin**
- **Integrin**

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### Strategies for reprogramming somatic cells

- **Somatic cell nuclear transfer / cell fusion approach**
- **Transcription-factor-based genetic approach**
- **Chemical approach**
  - The logic for small molecule strategy:
    - Identify small molecules replacing TFs
    - Combine those small molecules

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### Small molecules replacing TFs

- **Functionally replace transcription factors**
- **Enhance efficiency and accelerate speed in programming**
- **Reveal underlying mechanisms**
  - Epigenetic mechanisms through direct modulation of HMT, HDAC, HDM, DNMT, PRMT
  - Signaling mechanisms: MAPK inhibition, Ca^2+ / cAMP, Wnt, TGFβ inhibition / MET, Rho-ROCK inhibition

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PS48, sequentially combined with three other small molecules, enables human iPSCs using only Oct4

Zhu et al., Cell Stem Cell, 7, 651-655, 2010

Full developmental potentials of human Oct4-iPSC lines

Zhu et al., Cell Stem Cell, 7, 651-655, 2010

PS48 facilitates a metabolic switch during reprogramming

Factors

- PS48 is an allosteric activator of PDK1
- GFs-RTKs-PDK1-AKT signaling pathways
- cMyc
- Hypoxia-HIF pathway

Zhu et al., Cell Stem Cell, 7, 651-655, 2010

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PS48's reprogramming effect depends on glycolysis

PS48 acts on early stage of reprogramming

Zhu et al., Cell Stem Cell. 7, 651-655, 2010

PS48 induces glycolysis

Other metabolism modulators have similar reprogramming effects

A new paradigm for trans-differentiation

Cell-activation signal-directed (CASD) reprogramming

Approach:
- Transient expression of iPSC-factors
- Using lineage-specific soluble signals
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Direct reprogramming of fibroblasts to cardiac cells by CASD strategy

- Rep Media (+ JAK inhibitor, -LIF)
- Chemically-defined media (N2/B27, + BMP4)

Mid-stage reprogrammed cells express cardiovascular progenitor markers
Late-stage reprogrammed cells express mature cardiomyocyte markers

Cardiac mesoderm progenitor cells are generated during reprogramming

Contracting cardiomyocytes exhibit functional properties

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Pluripotency markers are not expressed during cardiac reprogramming

NanogMlc2aMesp1
Relative fold expression

Direct reprogramming of fibroblasts to induced neural progenitor cells/iNPCs

Expanded iNPCs can be further differentiated into neuronal and glial cells

Differentiated neurons exhibit functional properties

Evoked action potentials

Fast Na currents

Spontaneous EPSCs

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Pluripotent intermediate is not generated during iNPC process

Reprogramming fibroblasts to definitive endoderm and pancreatic precursor cells

Induced definitive endoderm cells can further differentiate into pancreatic progenitors
Further differentiation into pancreatic beta-like cells

In vivo transplantation of pancreatic progenitor cells/PPLC in STZ-induced diabetic mice

Transplanted pancreatic precursor cells could mature in vivo into all three pancreatic lineages, including functional insulin-secreting β-like cells

Reprogramming human fibroblasts into endothelial cells/iEnd cells

- Fibroblasts
- iEnd cells
- CD31/DAPI
- LDL uptake
- VE/DAPI
- Tube formation
- vWF/DAPI
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31. iEnd cells increased capillary density and improved perfusion in a mouse model of peripheral arterial disease

32. The CASD reprogramming model

33. Compared to conventional trans-differentiation

The CASD paradigm:
- A single TF combination for various cell types
- Transient TF expression could be more easily replaced
- Generate lineage-specific precursor cell type:
  - Which can be isolated, expanded, and characterized
  - May be more desirable for transplantation
  - Multi-potential
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Rapid induction and long term expansion of primitive NPCs from hESCs

By GS3 inhibitor + TGFβR inhibitor + hLIF

Homogenous self-renewal of primitive NPCs

Maintained high neurogenic propensity after expansion

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In vivo engraftment

DA neuron induction from long-term expanded primitive NPCs

Motor neuron induction from long-term expanded primitive NPCs
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CD4 T cell lineage differentiation

Ursolic acid specifically inhibit Th17 differentiation and ameliorated EAE disease in mice

Ursolic acid functions as a specific antagonist of RORyt to inhibit Th17 differentiation

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Thank you