Morphological understanding of beta-cell development and Regeneration

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Pancreatic endoderm
E8→E9

Pancreas budding

Gut rotation and fusion
Wandzioch & Zaret, Science 2009

3-4 S

Ventral pancreas

FGF-MAPK

TGF

BMP

FGF

HNF6

HNF1

Pdx1

alb1

Prox1

liver

5-6 S

Ventral pancreas

FGF-MAPK

TGF

BMP

FGF

HNF6

HNF1

Pdx1

alb1

Prox1

liver

Septum transversum mesenchyme
Foregut endoderm

- Specification
  - Hlxb9, Ptf1a, Tcf2, Hnf6, Hes1
  - Pdx/Ptf1a

Pancreas

- Growth
  - Pdx1, Rbpsuh, Hes1, Delta1, Fgf10
  - Pdx1
  - Hnf6

- Differentiation
  - Ngn3, Isl1, NeuroD, Insm1, Pax6, Nkx2.2, Nkx6.1, Pax4, Arx, Hlxb9, Foxa2
  - Ptf1a
  - Hnf6

Mature pancreatic cell types

- Acini
- Ducts
- Islets
Foregut endoderm

Hlxb9, Ptf1a, Tcf2, Hnf6, Hes1

Pdx/Ptf1a

Pancreas

Pdx1, Rbpsuh, Hes1, Delta1, Fgf10

Pdx1

Hnf6

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Acini, Ducts, Islets

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- Mature pancreatic cell types
Endocrine progentior

Ngn3

Isl1 NeuroD Insm1

Pax6 Pax4 Nkx2.2 Nkx6.1 Hlxb9

Pax6 Nkx2.2 Arx Foxa2

Pax6 Pax4

ε-cell (ghrelin+)

PP-cell

β-cell (insulin+)

α-cell (glucagon+)

δ-cell (somatostatin+)
Pancreatic injury and regeneration

In human, injured pancreatic tissues are hardly regenerated.

Young diabetic patients (T1D) with insulin pump

Pancreatic regeneration is important for beta cell replacement strategy.
Pancreatic neogenesis is a developing process of exocrine and endocrine tissues from common precursor cells in duct epithelium like embryonic organogenesis.
NEOGENESIS from pancreatic stem cells

Stem/progenitor cells

Proliferation /differentiation

Molecular factor for regeneration ?
NEOGENESIS from stem cells and transdifferentiation

ESC
Adult stem cell
Islet cell
Pancreatic duct cell
Pancreatic acinar cells

ESC → Islet cell → Pancreatic duct cell → Pancreatic acinar cells

Hnf6
Hes1, Delta1, Fgf10

Pdx1

Pdx/Ptf1a

Ptf1a

Mature pancreatic cell types
Acini
Ducts
Islets

Foregut endoderm

Hlxb9, Ptf1a, Tcf2, Hnf6, Hes1

Hlxb9, Ptf1a

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Endocrine cells

Pancreatic duct

Acinar cell

Endocrine cells

β-cell

Beta cell neogenesis by pancreatic cell transdifferentiation
Acinar cell

Ngn3
Pdx-1
MafA

β-cell

Acinar cell

Endocrine cells

β-cell

α-cell

β-cell
Endocrine cells

β-cell

Replication

β-cell

Nir et al., J Clin Invest. 2007
Dor et al., Nature, 2004
Endocrine cells

α-cell

β-cell
Endocrine cells

\[\alpha\text{-cell}\]

\[\beta\text{-cell}\]

DTR expression on islet β-cells

Wild type → No effect

Mosaic → ~50% β-cell ablation (hemizygous females)

Total → >99% β-cell ablation (males and homozygous females)

Insulin treatment

Glycemia (mmol/l)

DT

0 1 2 3 4 5 6 7 8 9 10
(months)

Diabetic

Recovered

Thorel1 et al., Nature 2010
Endocrine cells

α-cell

β-cell

Thorel1 et al.,
Nature 2010
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Pdx1</td>
<td>baseline</td>
<td>baseline</td>
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<tr>
<td>Ngn3</td>
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<td>MafA</td>
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<tr>
<td>Ins2</td>
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</table>

Thorel1 et al., Nature 2010
Beta cell generation types from various islet injuries

- Normal glucose homeostasis
- Faint or no regeneration (hemizygous RIP-DTP females; not shown)
- Glucose intolerance
- Regeneration by β-cell replication (nir, 07; Wang, 08; Cano, 08)
- Diabetes
- Regeneration from heterologous origins
Ectopic proteins for pancreatic regeneration

Clusterin
Nestin
INGAP
1. What are the crucial regulators in pancreatic regeneration in animal models

Pancreatic neogenesis is a developing process of exocrine and endocrine tissues from common precursor cells in duct epithelium like embryonic organogenesis.

Bioactive molecules expressing during the pancreatic neogenesis?

- Clusterin
- Nestin
- INGAP
- Transcription factors
Pancreatic regeneration in animal models

Different expression site of nestin and clusterin → functional diversity
Clusterin structure

Putative Function of Clusterin

- Lipid transportation
- Cytoprotection
- Cell-cell aggregation & interaction

Clusterin structure and function diagram.
Pancreas Regeneration In Clusterin KO Mice Model

Subtotal pancreatectomy → Regeneration from pancreatic remnant? → Recovery to normal glucose homeostasis?
Impaired pancreatic regeneration in CLU−/− → insulin deficiency
Impaired beta cell regeneration owing to absence of clusterin
Results in sequential response of insulin deficiency → hyperglycemia
& glucose intolerance → diabetes
In-vitro β-cell differentiation by clusterin

Duct stem/progenitor cells → Clusterin-overexpression/Clusterin protein → β-cell transformation
Beta-cell differentiation by Clusterin overexpression

Sub-total Px for pancreas regeneration

Isolation of duct stem cell

pcDNA3-CLU + pGFP vector

pcDNA3-CLU

GFP/DAPI

pcDNA3

pcDNA3-rCLU

Insulin cells/duct cells (10^3)

Insulin

pcDNA3

pcDNA3-rCLU

Non-TP  pcDNA3  pcDNA3-rCLU

42kDa

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pcDNA3 + pGFP vector

Insulin cells/duct cells (10^3)

Insulin

pcDNA3

pcDNA3-rCLU

Non-TP  pcDNA3  pcDNA3-rCLU

42kDa

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Beta-cell differentiation by Clusterin overexpression

Sub-total Px for pancreas regeneration

Isolation of duct stem cell

pcDNA3-CLU + pGFP vector

Insulin concentration (ng/ml)

Glucose concentration (mmol/l)

**

*Graph showing the relationship between insulin concentration and glucose concentration.*
Nestin

* Class VI intermediate filament protein (Lendahl et al., 1990)
* Neuroepithelial stem/progenitor cells in CNS (Lendahl et al., 1990)
* Neural stem cells in the brain (Morshead et al., 1994)
* Multipotential nestin-positive stem cells in pancreas (Zulewski et al., 2001, Kim et al., 2004)
Fig. 1
Nestin depletion and beta cell differentiation

Nestin positive stem cell

Nestin-null cells

Insulin-secreting beta-cell

Other cell phenotype

Pancreatic duct-stem cell culture
Differentiation into Insulin-Secreting Cells by Nestin RNAi

Nestin (+) vs. Nestin (-)

Fold change

- 0.0
- 0.2
- 0.4
- 0.6
- 0.8
- 1.0
- 1.2

*Mock siNestin

Insulin mRNA

Number of Insulin cells /PDS cells (10^3)

- Mock
- siNestin

*Insulin (ug/ml)

**Mock**

**siNestin**
Figure 5

**Mock**

**siNestin**

**ESC**

**PDSC**

**A)**

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
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<tbody>
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<td><strong>PCNA/PI</strong></td>
<td><strong>PCNA/PI</strong></td>
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**B)**

<table>
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<tr>
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<th>(+)</th>
<th>(-)</th>
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<th>(-)</th>
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<td>1.2</td>
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<tr>
<td>PDSC</td>
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**Fl-1 fluorescence intensity (normalized to control)**

**Counts**

- 0 to 40
- 60 to 120
- 160 to 220

**Fig. 5**
Mid EB

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mock</th>
<th>siNestin</th>
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<tbody>
<tr>
<td>HNF3β</td>
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<td>Isl-1</td>
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<td>Insulin</td>
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<tr>
<td>Glucagon</td>
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<td>Somatostatin</td>
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<td>Glut-2</td>
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<td>PC2</td>
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<tr>
<td>GAPDH</td>
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</tbody>
</table>

A) Conventional PCR  B) Real-time PCR

Fig. 6
Mock
siNestin
HNF3β
GATA4
Pdx-1
Ngn3
Pax4
Isl-1
Insulin
Glucagon
Somatostatin
Glut-2
PC2
GAPDH

Fig. W7
A) Conventional PCR
B) Real-time PCR

x-fold induction / control

0.0 0.2 0.4 0.6 0.8 1.0
Fig. 8

- **A)** Conventional PCR
- **B)** Real-time PCR

Gene expressions for Mock and siNestin:
- Pdx-1
- Ngn 3
- Nkx 2.2
- Isl-1
- Insulin
- Glucagon
- GAPDH

Histogram showing x-fold induction/control values for Mock and siNestin conditions.
Conclusion

Profiles of pancreatic regeneration shares important morphological feature and molecular regulation with embryonic organogenesis of pancreas
Acknowledgement

Thank you for attention!