通过转分化实现胰岛细胞的再生

李维达 同济大学

器官组织再生

Lazzaro Spallanzani

1768-Regeneration

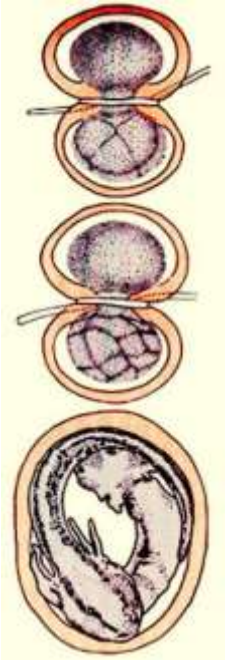
(http://en.wikipedia.org/wiki)

(Image: AAAS/Science)

人类具有有限的再生能力

婴儿头发的传奇

Hans Spemann (1869 –1941)



1. 用婴儿头发将娃娃鱼受精卵分成两部分，有核那部分继续发育。

2. 在**16**细胞期，把一个已经分化的核推回无核的那部分。

3. 被推过去的细胞核去分化，发育成另外独立的娃娃鱼。

第一个揭示细胞可以被再重编**(reprogramming)**的实验。

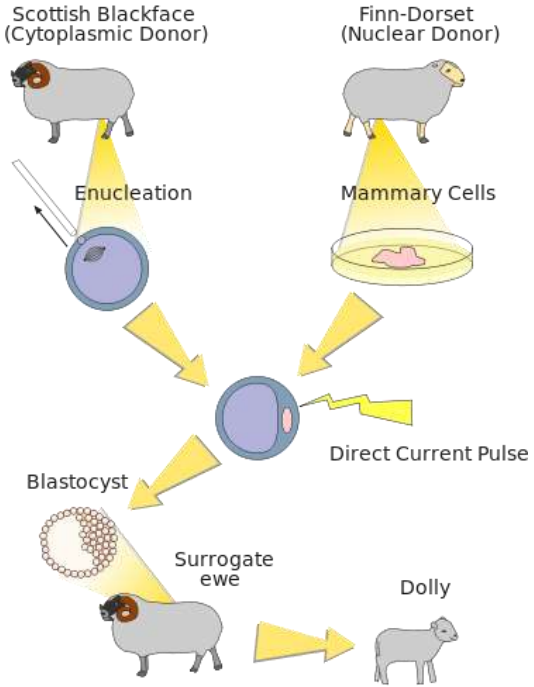
体细胞克隆

Sir John B. Gurdon

约翰—格登（英国）

1962 clone frogs

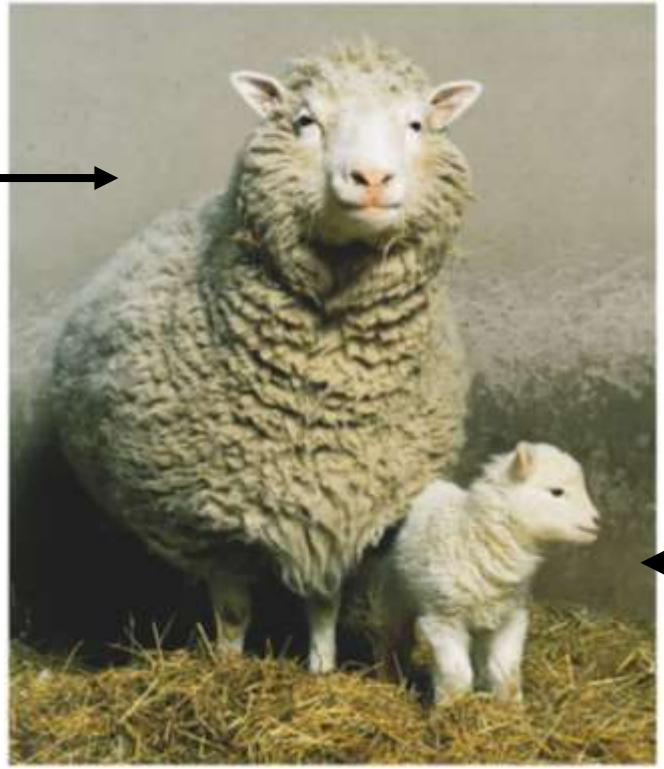
体细胞克隆-多莉的诞生



http://en.wikipedia.org/wiki/Dolly_the_sheep

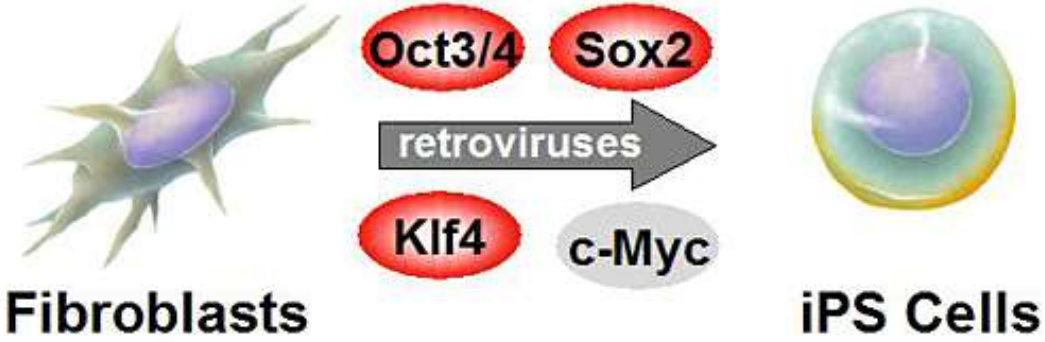
Dolly 和她的女儿 Bonnie

Dolly (1996-2003)



Bonnie

Induced Pluripotent Stem (iPS) Cells



Mouse iPS cells reported in 2006

Human iPS cells reported in 2007

细胞重编程-2012 诺贝尔生理学奖

Sir John B. Gurdon

约翰—格登（英国）
1962 克隆蟾蜍

Shinya Yamanaka

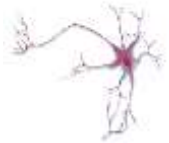
山中 伸弥（日本）
2006 诱导性多能干细胞

ES/iPS

外胚层


皮肤


脑组织


神经组织

中胚层


血液


心脏


肾脏


肌肉

内胚层


肺脏

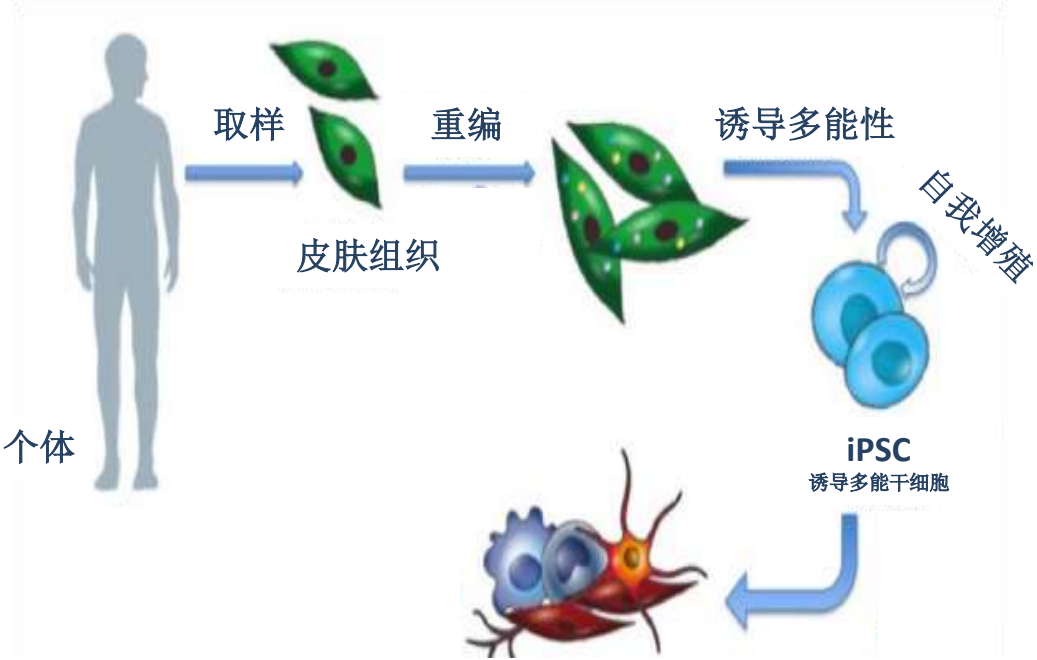

肝脏


胃


肠

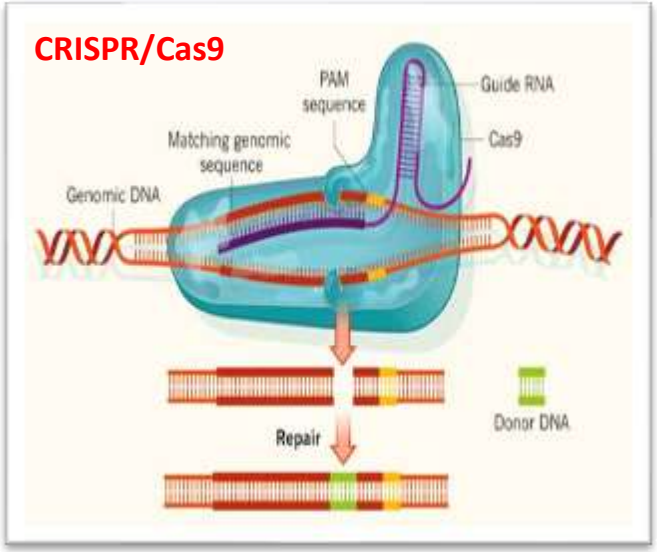

胰腺

干细胞技术与细胞再生



适合每一个人的组织器官

基因编辑

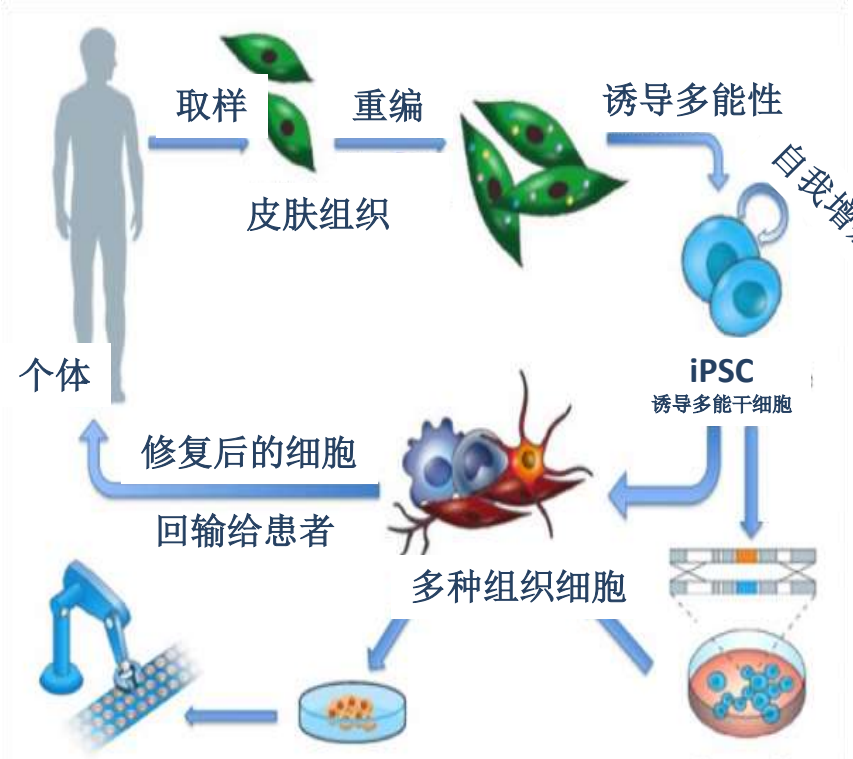


DNA 双链结构非常稳定保证生命信息的高保真复制，但是也难以用传统方法编辑修复。

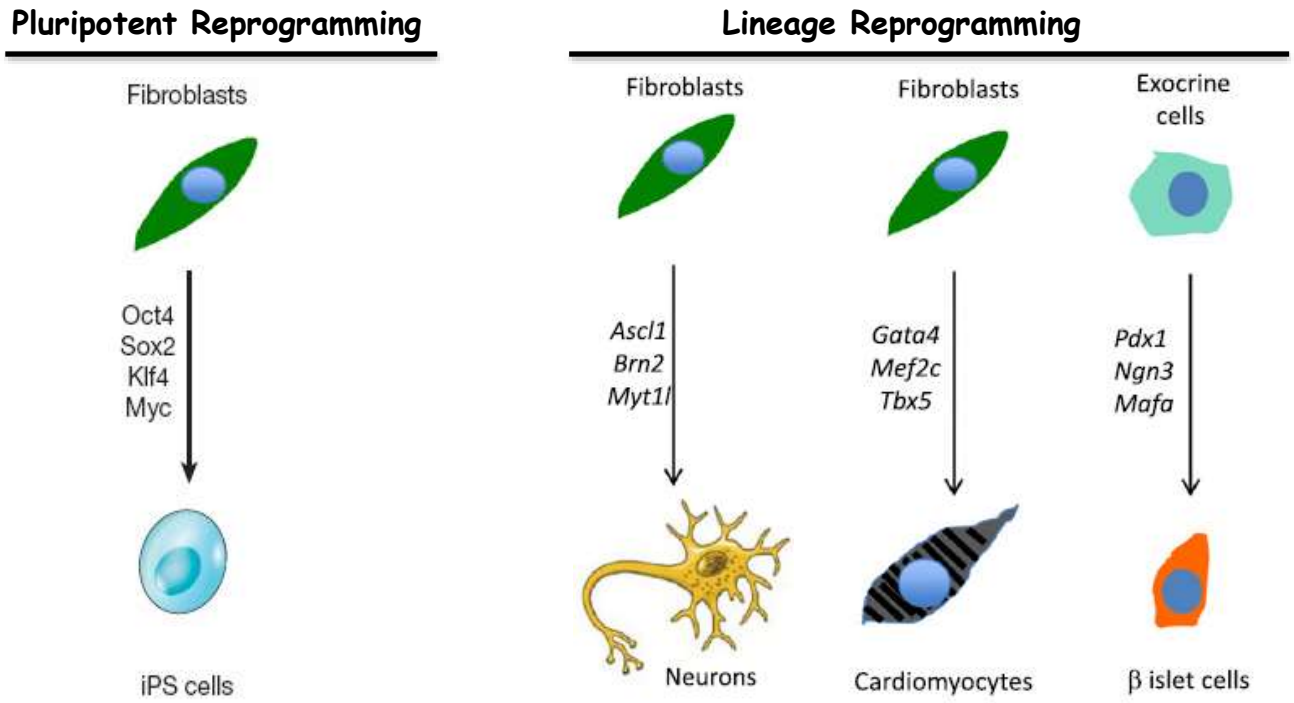
CRISPR/Cas9技术解决基因编辑两大难题：

1. 怎样切？
2. 在什么地方切？

干细胞技术 + 基因编辑技术 = 精准医疗平台



谱系重编程（转分化）



In vivo transdifferentiation

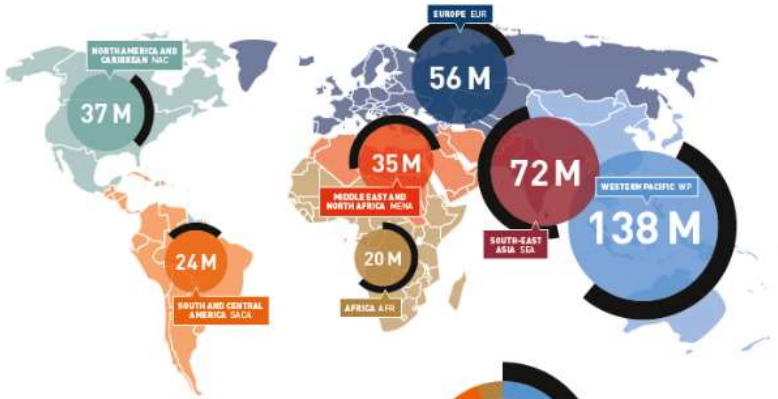
Modified from Thomas Graf, Cell Stem Cell (2011)

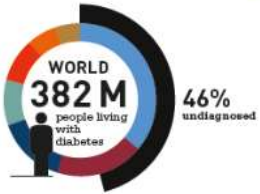
关键科学问题

- Whether pancreatic reprogramming can be applied as a cell replacement therapy to cure diabetes?

- How to regenerate every specific cell type of the mini-organ islet?

糖尿病的危害

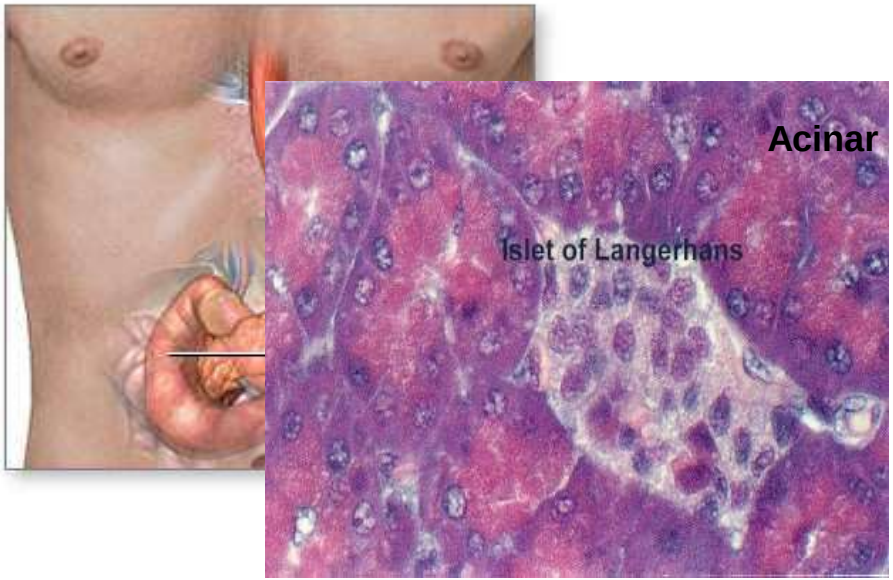




Huge burden of medical care for diabetes

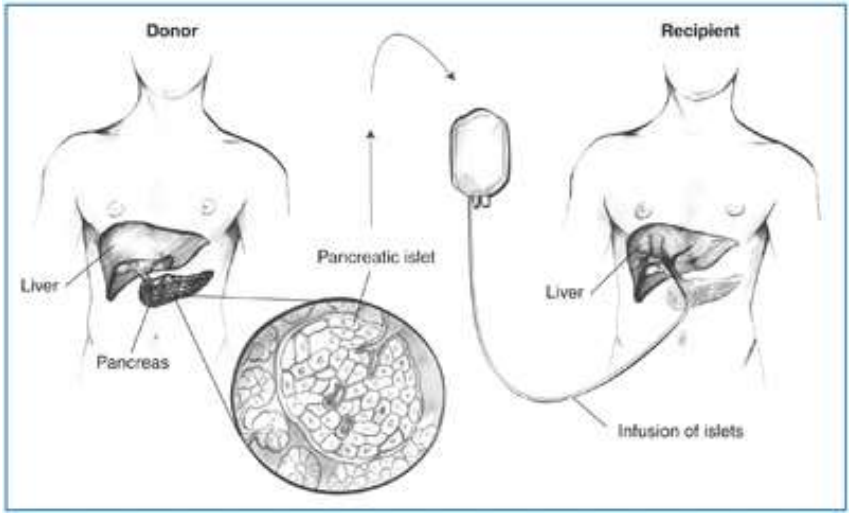

Lost Eyesight & Blindness
Dialysis & Kidney Failure
Sores & Amputation

糖尿病中的胰岛细胞缺损



- Diabetes: ~11.6 % in Chinese adult population
- Type 1 diabetes: autoimmune destruction; β-cell loss
- Type 2 diabetes: insulin resistance; β-cell loss
- Insulin injection is not a cure, causing hypoglycemia

胰岛移植

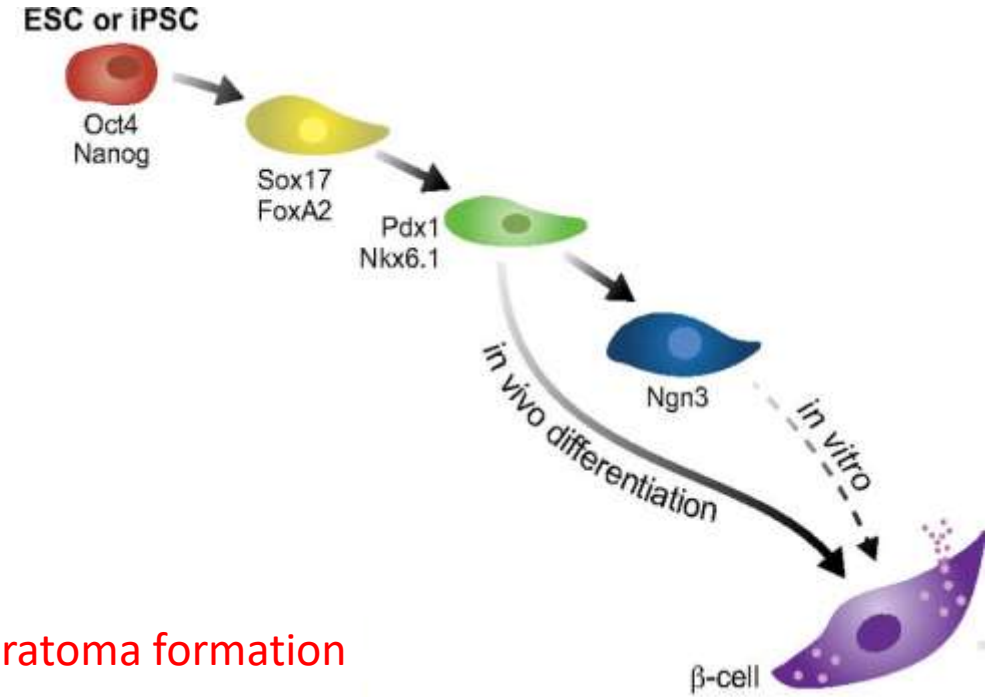


http://diabetes.niddk.nih.gov/dm/pubs/pancreaticislet/

Problems:

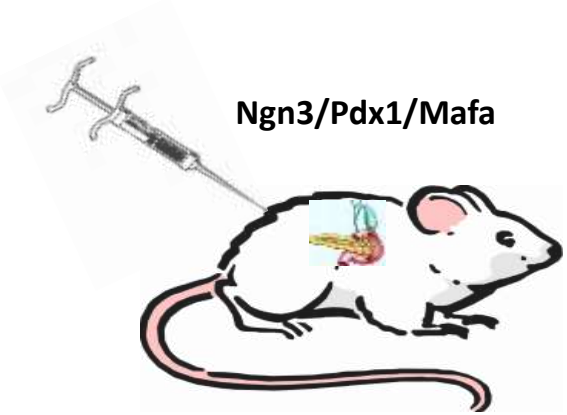
Shortage of islets	+	Immunosuppressive drugs

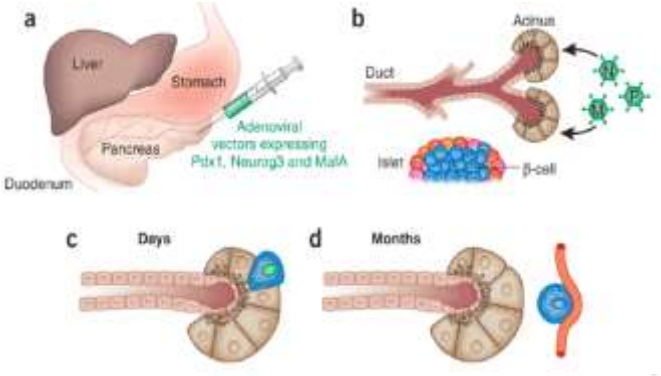
从多能干细胞诱导分化而来的胰岛β细胞



Risk: teratoma formation

胰腺转分化再生胰岛β细胞



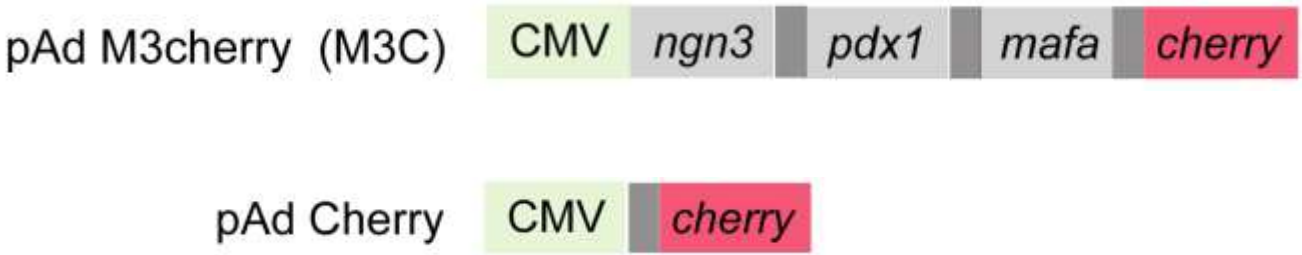


***In vivo* reprogramming via surgery**

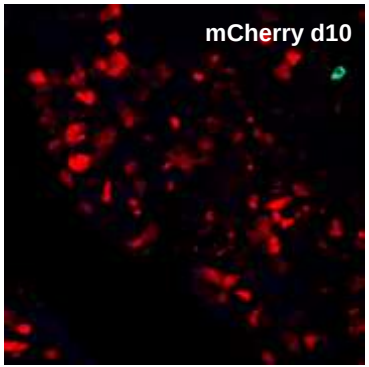
Zhou et al, Nature (2008)

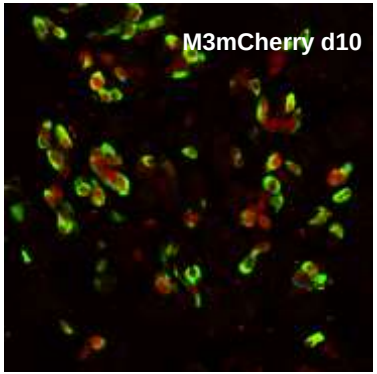
- A potential approach for cell replacement therapy for diabetes.
- However, the efficiency is low and induced beta cells lack long-term stability .

重编程因子串联表达系统

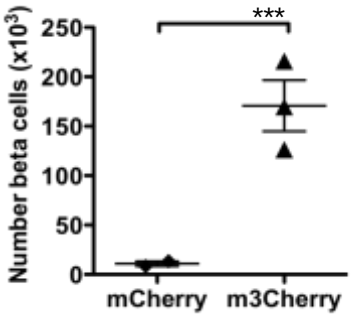


Insulin / mCherry





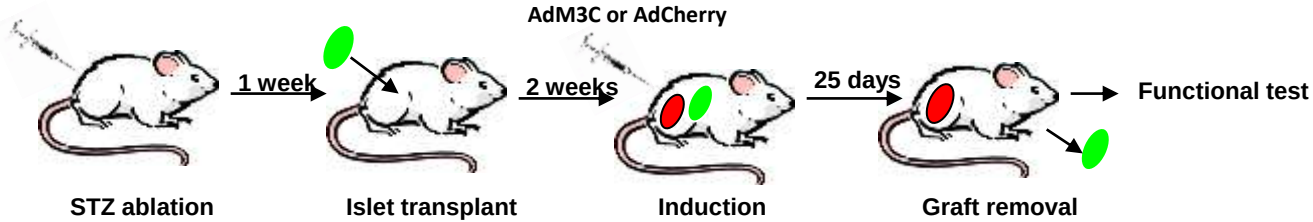
Induced β cells No.



Insulin expression

Day 10

糖尿病模型中检测转分化再生胰岛β细胞功能

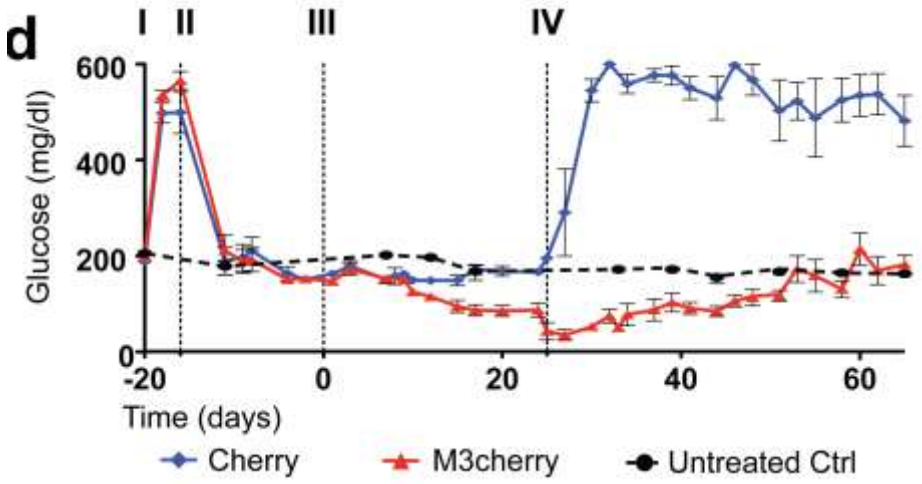


In collaboration with Gordon Weir Lab at Joslin Diabetes Center

胰岛β细胞功能检测指标

- Secrete insulin and regulate glycemic condition
- Know when to release insulin: responsive to glucose stimulation
- Know when to shut off: sensitive to low glucose levels

回复高血糖



I STZ

II Islet transplant

III Induction

IV Graft removal

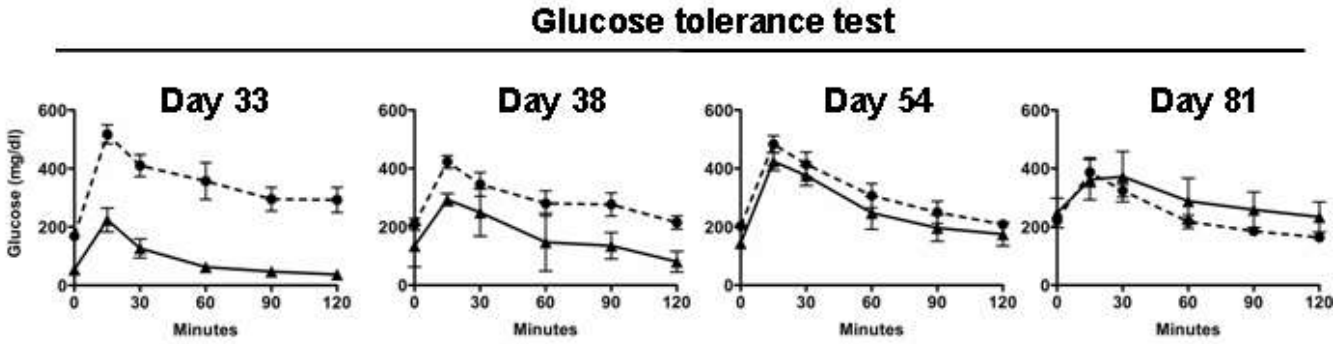
Insulin expression

Day 10

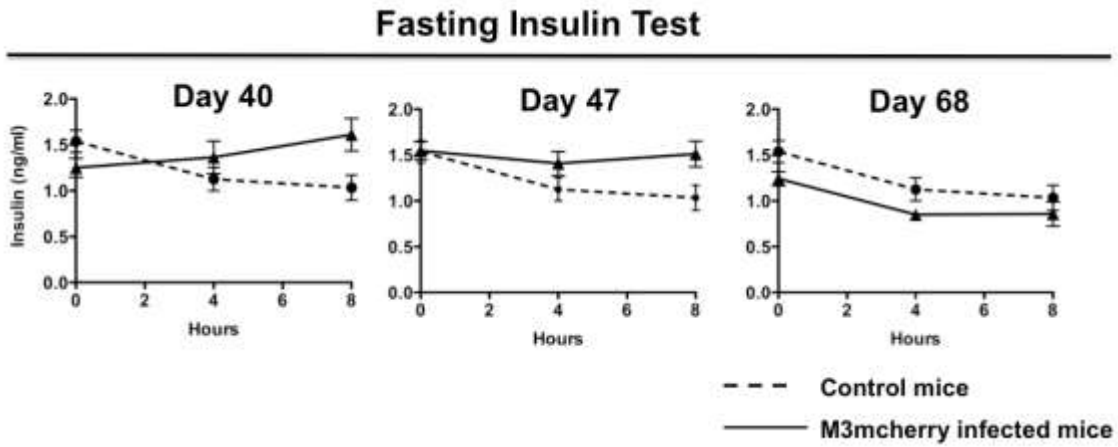
Gain ability to regulate glycemia

Day 30

对高血糖敏感


Glucose tolerance test
Day 33
Day 38
Day 54
Day 81
Glucose (mg/dl)
600
400
200
0
0
30
60
90
120
Minutes
Control mice
M3mcherry infected mice

对低血糖敏感



Insulin expression

Day 10

Gain ability to regulate glycemia

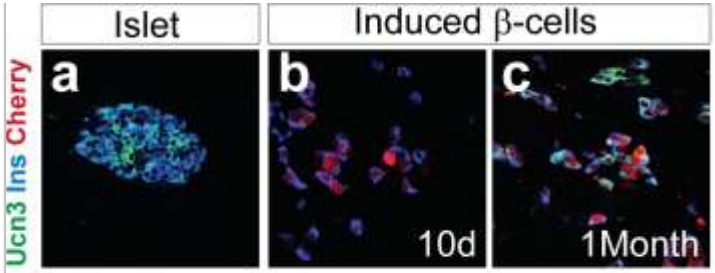
Day 30

Respond normally to glucose level changes

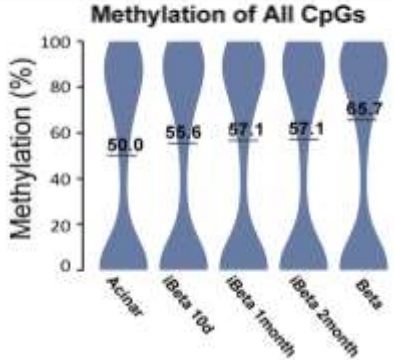
Day 60

转分化而来的β细胞的转录组和表观遗传上的变化

Transcriptional evolvement

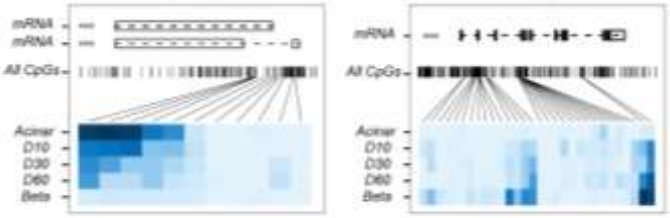


Epigenetic evolvement



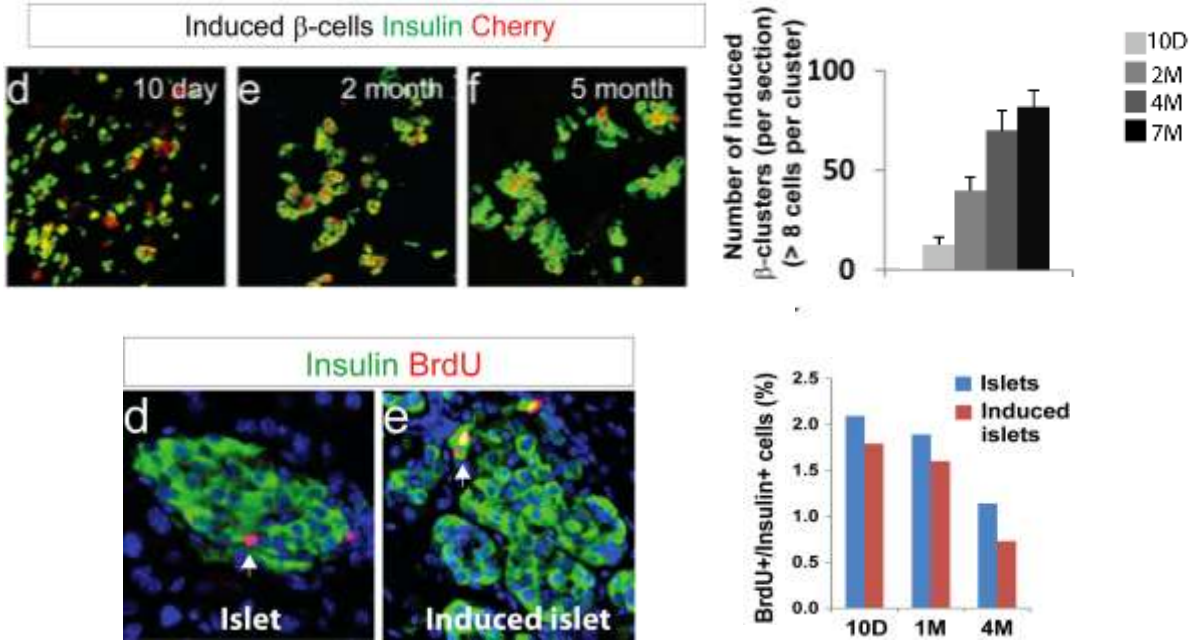
β cell-Kcnj11

Acinar cell-RbpjL



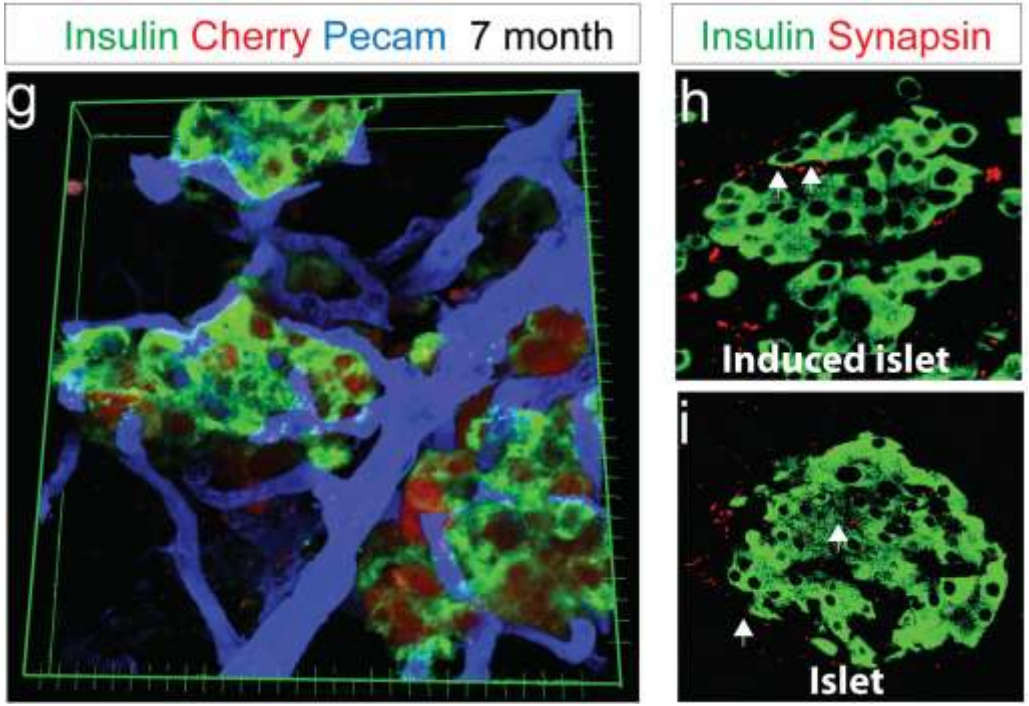
In collaboration with Alex Meissner Lab

类胰岛结构的形成

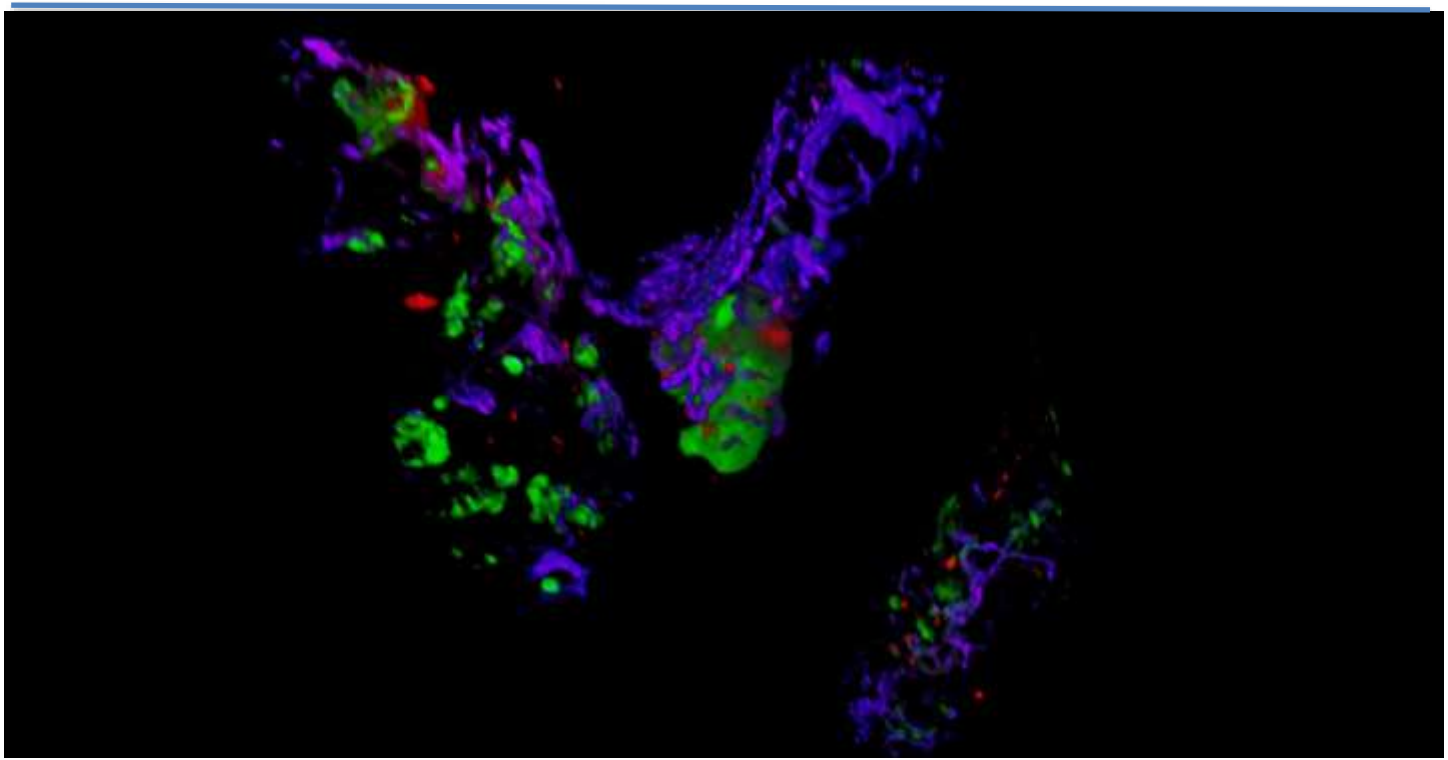


β cell migration and aggregation might be the drive for islet formation.

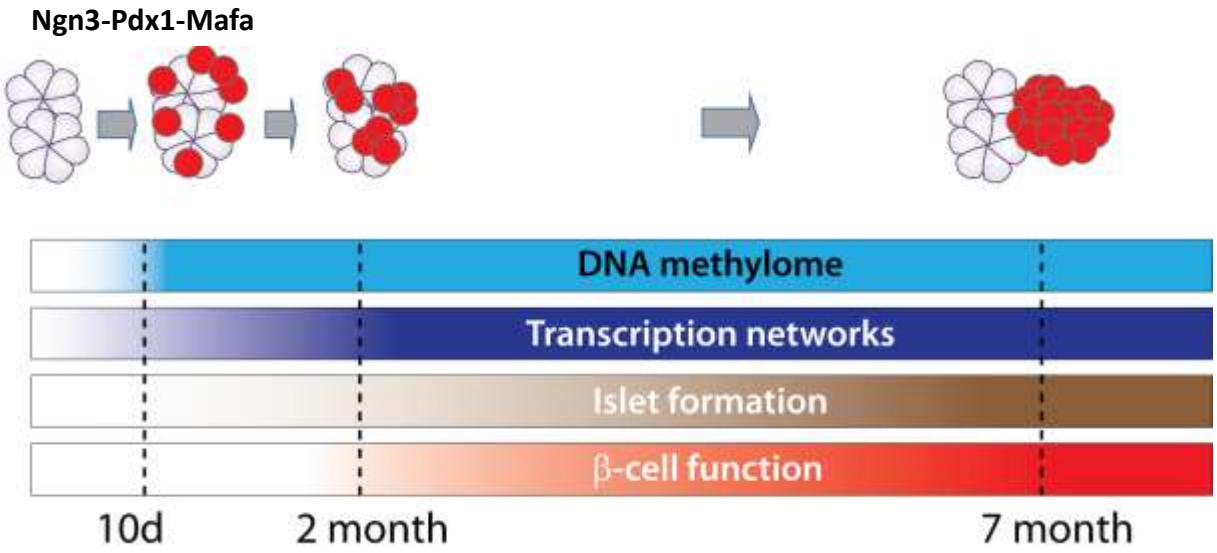
类胰岛结构与血管神经联结



类胰岛结构3D成像



总结 I



(Li W et al., Nature Biotechnology, 2014)

关键科学问题

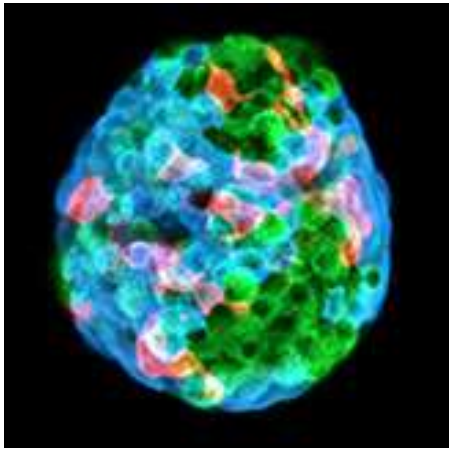
- Whether pancreatic reprogramming can be applied as cell replacement therapy to cure diabetes?

  Induced β cells undergo a maturation process to obtain full function.
  This maturation includes epigenetic, transcriptional, and cellular evolvement.

- How to regenerate every specific cell type of the mini-organ islet?

- What is the molecular mechanism?

胰岛内三种主要胰岛细胞



Islet is a mini-organ.

Three major endocrine cells

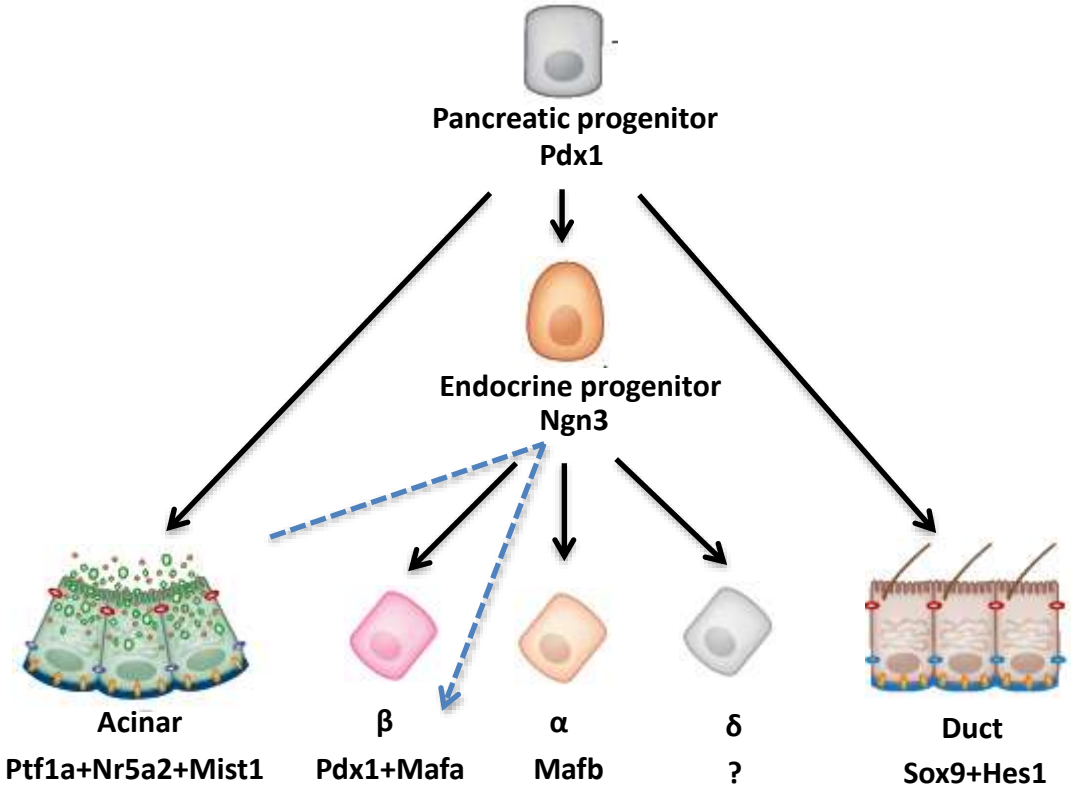
Beta (Insulin) ✔

Alpha (Glucagon) ***?***

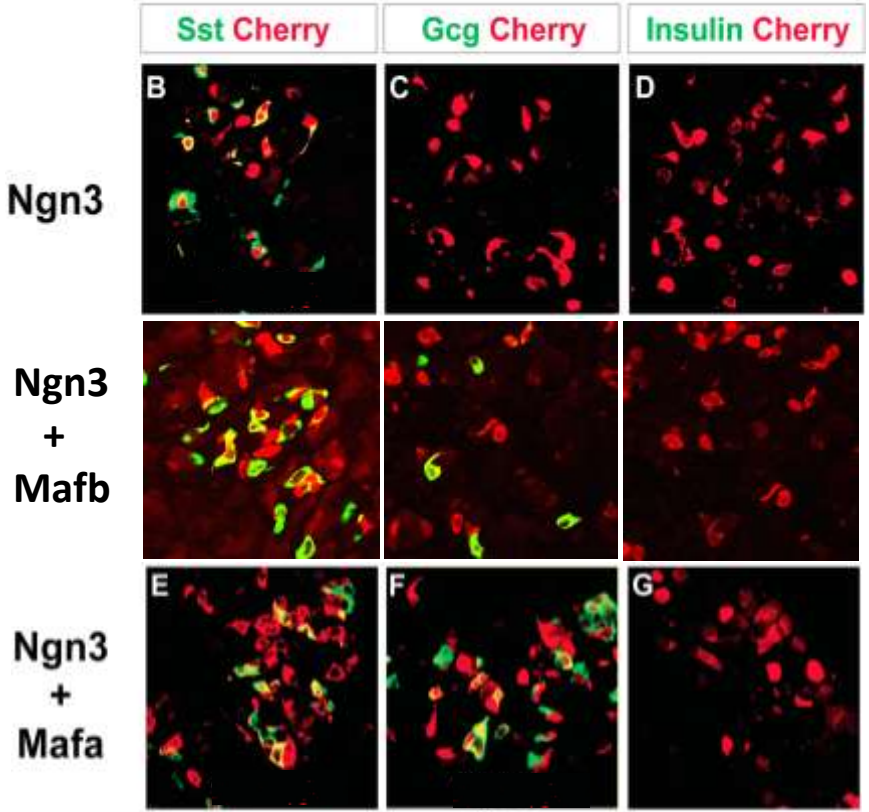
Delta (Somatostatin) ***?***

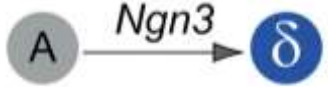
- Alpha cells produce the hormone glucagon to maintain plasma glucose levels by stimulating hepatic glucose production.
- Delta cells produce somatostatin to control the secretion of glucagon and insulin.

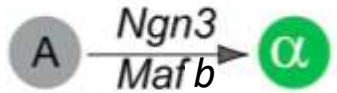
胰岛细胞的发育


Pancreatic progenitor
Pdx1
Endocrine progenitor
Ngn3
Acinar
Ptf1a+Nr5a2+Mist1
β
Pdx1+Mafa
α
Mafb
δ
?
Duct
Sox9+Hes1

转分化再生δ and α细胞

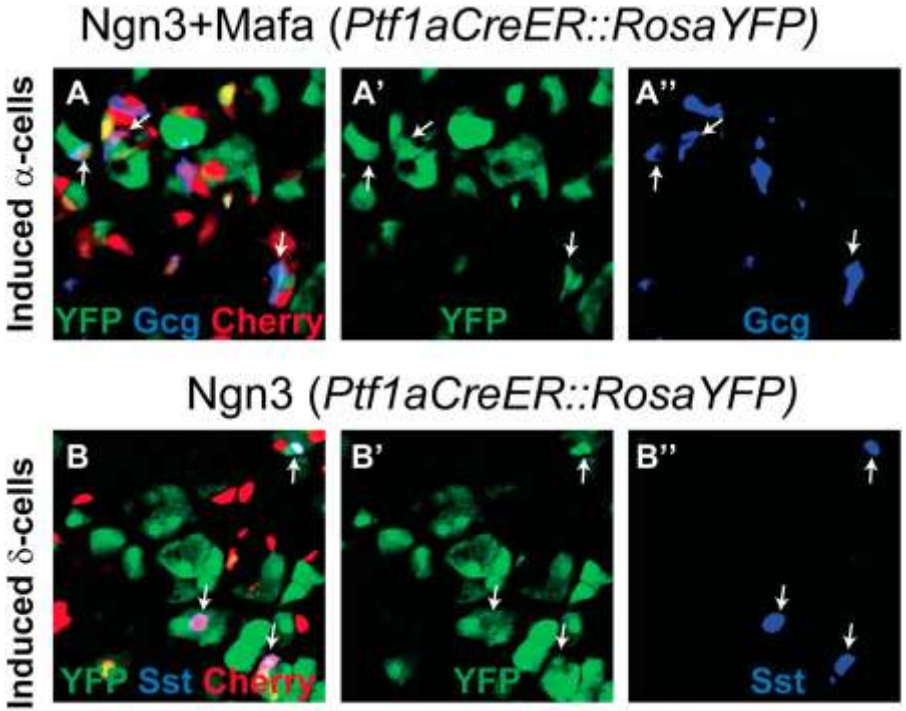




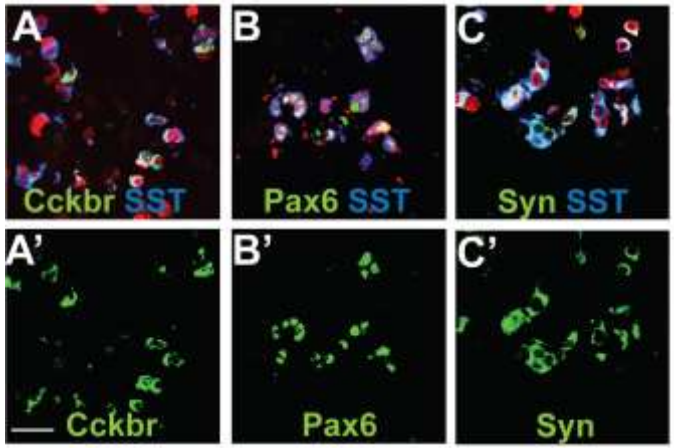


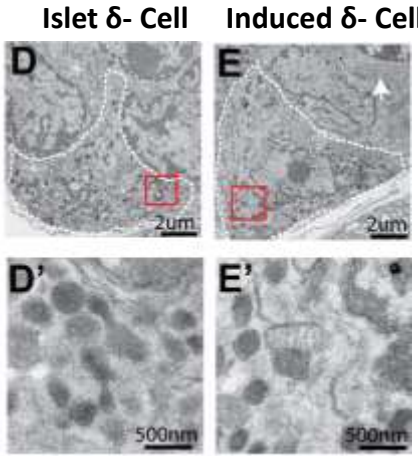
Low efficiency

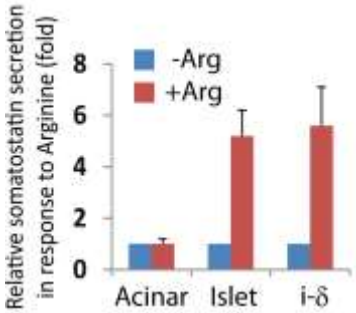
谱系追踪实验


Ngn3+Mafa (*Ptf1aCreER::RosaYFP*)
Induced α-cells
A
A'
A''
YFP Gcg Cherry
YFP
Gcg
Ngn3 (*Ptf1aCreER::RosaYFP*)
Induced δ-cells
B
B'
B''
YFP Sst Cherry
YFP
Sst

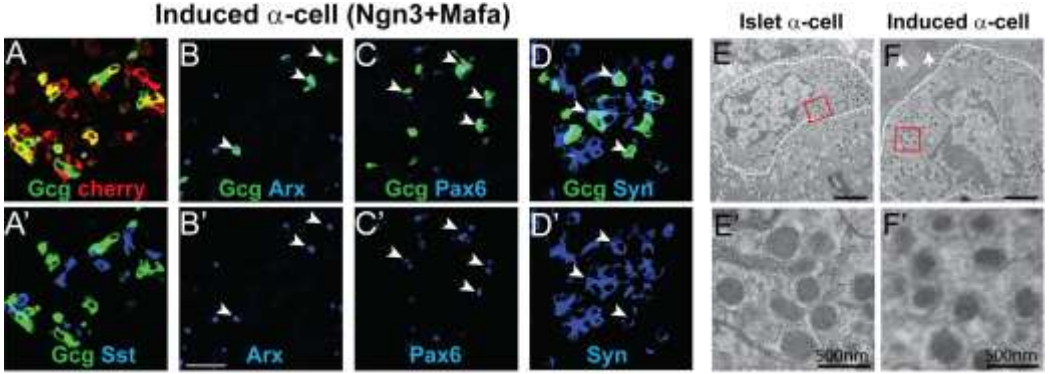
转分化而来的胰岛δ细胞

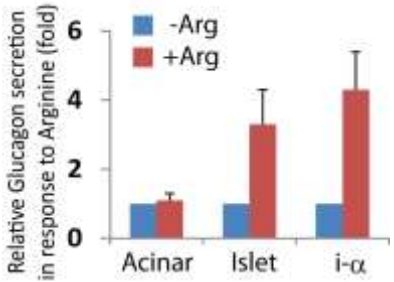




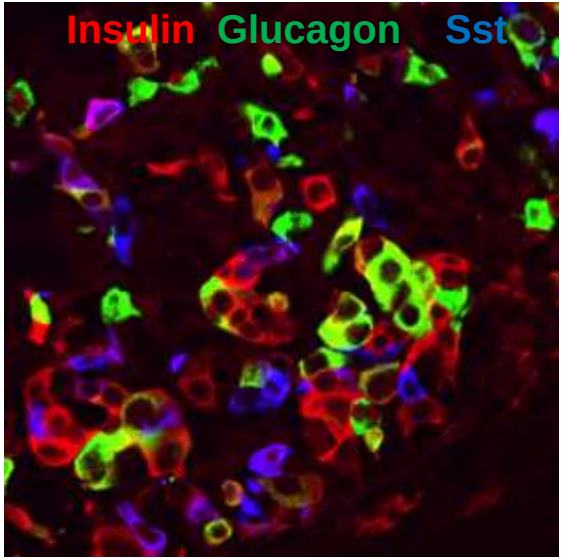


转分化而来的胰岛α细胞

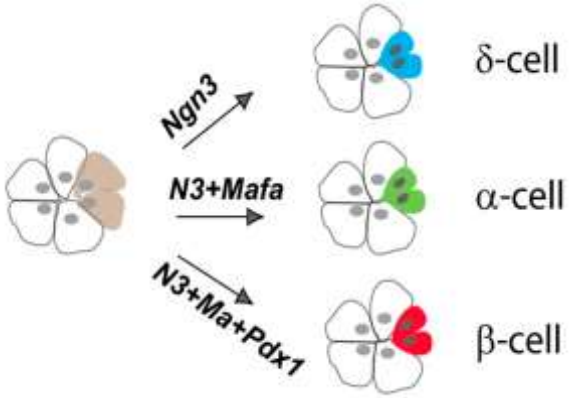




小结 II



Ngn3+Pdx1+Mafa



(Li W et al., eLIFE, 2014)

关键科学问题

- Whether pancreatic reprogramming can be applied as cell replacement therapy to cure diabetes?

  Induced β cells undergo a maturation process to obtain full function.
  This maturation includes epigenetic, transcriptional, and cellular evolvement.

- How to regenerate every specific cell type of the mini-organ islet?

  A simple code of reprogramming factors can direct conversion of acinar cells to all three major islet endocrine cell types *in vivo*.

课后讨论

1. 糖尿病分几类，各有何特点？
2. 糖尿病的危害及现行治疗手段？
3. 胰岛细胞再生的方式？
4. 比较转分化与分化再生胰岛细胞的优缺点。

Ultimate goal — to cure diabetes

We wanted to replace " insulin injections" with "nature's own solution."

Douglas Melton, Director of Harvard Stem Cell Institute


THANKS FOR
ATTENTION