The Use of Stem Cells as a Cure for Type 1 Diabetes

January 15, 2010
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In theory….

1. Pretty well worked out.

2. ~Easy and more options coming.

3. This is the issue!

1. Pretty well worked out.
Problems

• **Supply** versus demand.
• Huge loss of islets during purification.
• Adverse reaction to immunosuppression drugs, increased hypertension (6-42%), increase need for statin therapy (23-83%).
• After 1 year, 44% were insulin independent, 28% had partial graft function and 28% had complete graft loss after a year.
• After 5 years, 10% were insulin independent, 70% had partial graft function, 20% complete loss of graft.
In theory....

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Mouse Embryonic Stem (ES) cells

Derived from ICM of blastocyst
To generate ES cells

Isolate ICM

Feeder cells
Growth factors
Serum
Mouse Blastocysts in Culture
Figure 1 Colonies of undifferentiated ES cells growing on a layer of irradiated primary mouse embryo fibroblasts. Note the smooth outline of the colonies of densely packed cells. Differentiated cells adhere more tightly to the surface of the dish.
Embryonic Stem Cell Lines Derived from Human Blastocysts

James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, Jeffrey M. Jones

SCIENCE  VOL 282  6 NOVEMBER 1998
Everchanging Status of hES cell research in US.

Research with federal grants on human ES cells was restricted to the “78” lines “derived” as of August 9, 2001.

From http://www.news.wisc.edu/packages/stemcells/es_gpt.html
Everchanging Status of hES cell research in US.

NIH Human Embryonic Stem Cell Registry

In response to Executive Order 13505, issued on March 9, 2009, NIH has developed new guidelines to establish policy and procedures under which NIH will fund research in the area of human stem cells, and to help ensure that NIH-funded research in this area is ethically responsible, scientifically worthy, and conducted in accordance with applicable law. These Guidelines are effective on July 7, 2009.

Scientists may determine whether a particular hESC line is eligible for use in NIH-supported research under the new Guidelines by referring to the NIH Human Embryonic Stem Cell Registry.

- NIH Human Embryonic Stem Cell Registry.
Everchanging Status of hES cell research in US.

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<tr>
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Etc.....
Why do we need new stem cell lines?

Panels of cell lines for tissue matching in transplantation. Safety hazards with current cells derived using mouse feeders and serum.

**Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells**

Ren-He Xu¹², Ruthann M Peck¹, Dong S Li¹, Xuezhu Feng², Tenneille Ludwig² & James A Thomson¹²

Only 21 lines available, second generation ES lines have better properties, third generation probably even better?? There are other hES lines being made in Canada, Australia, Singapore and the UK but no one in US can use them with federal grant money.

So...this research is going on abroad, in CA, MA, NJ, MD or in privately funded efforts....
Murine ES cells can differentiate *in vitro*, form tumors and contribute to chimeras.
Gold standard--teratomas.
Could be a problem!!
Mouse AND Human iPS cell lines
(your Bioreg December FOCUS paper)

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi¹ and Shinya Yamanaka¹,²,*
¹Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
²CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,¹ Koji Tanabe,¹ Mari Ohnuki,¹ Megumi Narita,¹,² Tomoko Ichisaka, and Shinya Yamanaka¹,²,³,*
¹Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
²CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
³Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA
*Correspondence: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2007.11.019

Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

Junying Yu,¹,²,* Maxim A. Vodyanik,² Kim Smuga-Otto,¹,² Jessica Antosiewicz-Bourget,¹,² Jennifer L. Frane,¹ Shulan Tian,³ Jeff Nie,³ Gudrun A. Jonsdottir,³ Victor Ruotti,³ Ron Stewart,³ Igor I. Slukvin,²,⁴ James A. Thomson¹,²,⁵,*

These can be “customized”. Murine lines have been used to “correct” sickle cell disease already…. 
Complete Genetic Correction of iPS Cells From Duchenne Muscular Dystrophy

Step 1: Human chromosome 21
- Telomere seeding
- Human chromosome X
- HAC vector
- Chromosomal translocation with Cre
- DYS-HAC vector

Step 2: Fibroblasts with genetic defect
- Chromosome transfer (MMCT)
- Genetically corrected fibroblasts
- Induction of iPS cells with defined factors
- Genetically corrected iPS cells
- Chromosome transfer (MMCT)
- Induction of iPS cells with defined factors

Step 3: Differentiation to muscle stem cells in vitro
- Teratoma formation
  1. Differentiation to muscle cells
  2. Expression of human dystrophin

Step 4: Transplantation of genetically corrected autologous cells
- Fibroblasts with genetic defect
- iPS cells with genetic defect
- mdx mice (DMD model mice)
- DMD patient
Another technical advance.

Producing primate embryonic stem cells by somatic cell nuclear transfer

1. Tested mitochondrial DNA.
2. Tested “stemness” markers.
3. Differentiation ability in vitro.
4. Teratomas.
1. Pretty well worked out.

2. ~Easy and more options coming.

3. This is the issue!
Differentiation of Embryonic Stem Cells to Insulin-Secreting Structures Similar to Pancreatic Islets

Nadya Lumelsky,¹ Olivier Blondel,¹,³ Pascal Laeng,²,⁴ Ivan Velasco,¹ Rea Ravin,¹ Ron McKay¹*

SCIENCE  VOL 292  18 MAY 2001
Let's start with mouse:

Generation of insulin-secreting pancreatic islet clusters from undifferentiated mouse ES cells

Stage 1: (2-3 days)
Expansion of ES cells:
on gelatin-coated tissue culture surface without feeder cells and in the presence of LIF.

Stage 2: (4 days)
Generation of EBs:
in suspension, in ES cell medium in the absence of LIF.

Stage 3: (6-7 days)
Selection of nestin positive cells:
ITSFn medium on tissue culture surface.

Stage 4: (6 days)
Expansion of pancreatic endocrine progenitor cells:
N2 medium containing B27 media supplement and bFGF.

Stage 5: (6 days)
Induction of differentiation and morphogenesis of insulin-secreting islet clusters:
withdraw bFGF from N2 medium containing B27 media supplement and nicotinamide.

Ins+ cells associate with neurons

Cells contained Insulin at ~1ug/mg total protein

They transplanted these into streptozotocin-diabetic mice and they kinda sorta improved blood glucose levels.
Insulin Staining of ES Cell Progeny from Insulin Uptake

Jayaraj Rajagopal, William J. Anderson, Shoen Kume, Olga I. Martinez, Douglas A. Melton*

SCIENCE VOL 299 17 JANUARY 2003

BUT…..

Showed cells were not transcribing Insulin mRNA, ES lines with a Pdx1-lacZ or Insulin-lacZ did not turn blue, did not stain for C-peptide of Insulin, did not have granules (EM) and cells treated with FITC-Insulin took it up from the medium.
So the protocols got more complicated and the assays more rigorous.
So the protocols got more complicated and the assays more rigorous but we’re still far away from a β cell
Who knows best how to make a pancreas and/or a β cell?

Gastrulation and Formation of Definitive Endoderm
Pancreas Development

Caveat: This is just what we know about so far!!

**endoderm**
- induce Pdx1
- inhibit Shh
- induce Ptf1a

**duodenal/pancreatic precursors**
- inhibit Ptf1a
- induce Ptf1a

**duodenal mucosa & enteroendocrine cells**
- induce Ngn3

**pancreatic precursors**
- decrease Pdx1
- maintain Ptf1a

**endocrine cells**
- α
- β
- δ
- PP

**exocrine cells**
- increase Pdx1

*Slide courtesy of M. Gannon*
Production of pancreatic hormone–expressing endocrine cells from human embryonic stem cells

Kevin A D’Amour, Anne G Bang, Susan Eliazer, Olivia G Kelly, Alan D Agulnick, Nora G Smart, Mark A Moorman, Evert Kroon, Melissa K Carpenter & Emmanuel E Baetge

VOLUME 24  NUMBER 11  NOVEMBER 2006  NATURE BIOTECHNOLOGY

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1–2 days 1–2 days 2–4 days 2–4 days 2–3 days 3+ days

ES ME DE PG PF PE EN

OCT4 NANOG SOX2 ECAD BRA FGF4 WNT3 NCAD SOX17 CER FOXA2 CXCR4 HNF1B HNF4A PDX1 HNF6 HLXB9 NKK6-1 NGN3 PAX4 NKK2-2 INS CGC GHRL SST PPY
endoderm

> duodenal/pancreatic precursors

induce Pdx1

inhibit Shh

endocrine cells

< pancreatic precursors

induce Ngn3

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CXCR4
HNF1B
HNF4A
PDX1
HNF6
HLXB9
NKX6-1
NGN3
PAX4
NKK2-2
INS
CGC
GHRL
SST
PPY
**a**

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- NNX6-1*
- NGN3
- PAX4
- NNX2-2 *
- INS *
- CGC
- GHRL
- SST
- PPY
BUT!!!
hES cells turn to the embryo!

Two issues….efficiency (low) and insulin response to glucose (they don’t)…but it’s a fantastic step forward. They used 5 different cell lines and they all worked.
Original Research Report

Directed Differentiation of Human Embryonic Stem Cells into the Pancreatic Endocrine Lineage

BLAINE W. PHILLIPS,¹ HANNES HENTZE,¹ WILLIAM L. RUST,¹ QI-PING CHEN, HIRAM CHIPPERFIELD, EE-KIM TAN, SUMAN ABRAHAM, AKILA SADASIVAM, POH LOONG SOONG, SIEW TEIN WANG, RICKY LIM, WILLIAM SUN, ALAN COLMAN, and N. RAY DUNN

3D protocol using embryoid bodies in Matrigel

Pdx1 expression
Early markers come on first.
Then Pdx1 and Ptf1a and Ngn3.
Stem cell markers are gradually downregulated. Sox17 and Foxa2 come up on the periphery (primitive endoderm) and Pdx1 comes on late.
Pdx1 mRNA is in a restricted region (niche?) and Insulin mRNA is expressed.
Expression of markers for embryonic pancreas...and islet cells.
Mixed the differentiated cells with Matrigel and transplanted them into STX-SCID (diabetic) mice. And it sort of worked.
A newer protocol also based on understanding how the embryo makes a pancreas:

A small molecule that directs differentiation of human ESCs into the pancreatic lineage

Shuibing Chen¹,², Malgorzata Borowiak¹,⁴, Julia L Fox¹,⁴, René Maehr¹,², Kenji Osafune¹, Lance Davidow¹, Kelvin Lam¹, Lee F Peng³, Stuart L Schreiber³, Lee L Rubin¹ & Douglas Melton¹,²

Certainly getting closer....
In theory...

1. Pretty well worked out.
2. Patient specific cells. ✓
3. This remains an issue!

The Use of Stem Cells as a Cure for Type 1 Diabetes