Stem Cells and Therapies

Renee A Reijo Pera, PhD
Director and Professor
Center for Human Embryonic Stem Cell Research and Education
Institute for Stem Cell Biology & Regenerative Medicine

Outline

I. Introduction to stem cells

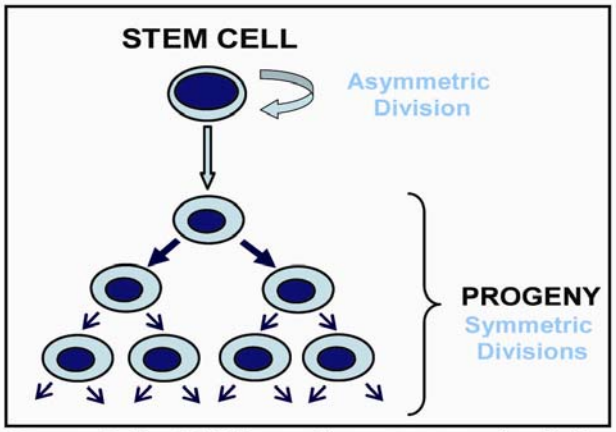
II. Human embryonic stem cells:
Development & derivation from human embryos

III. Alternative Methods: Reprogramming

IV. Stem cells and human health
A. Reproduction and Fetal Outcome
B. Somatic Health
C. Cancer Biology

V. Summary and Conclusions

I. Stem Cells: Cells That Can Self-Renew or Differentiate

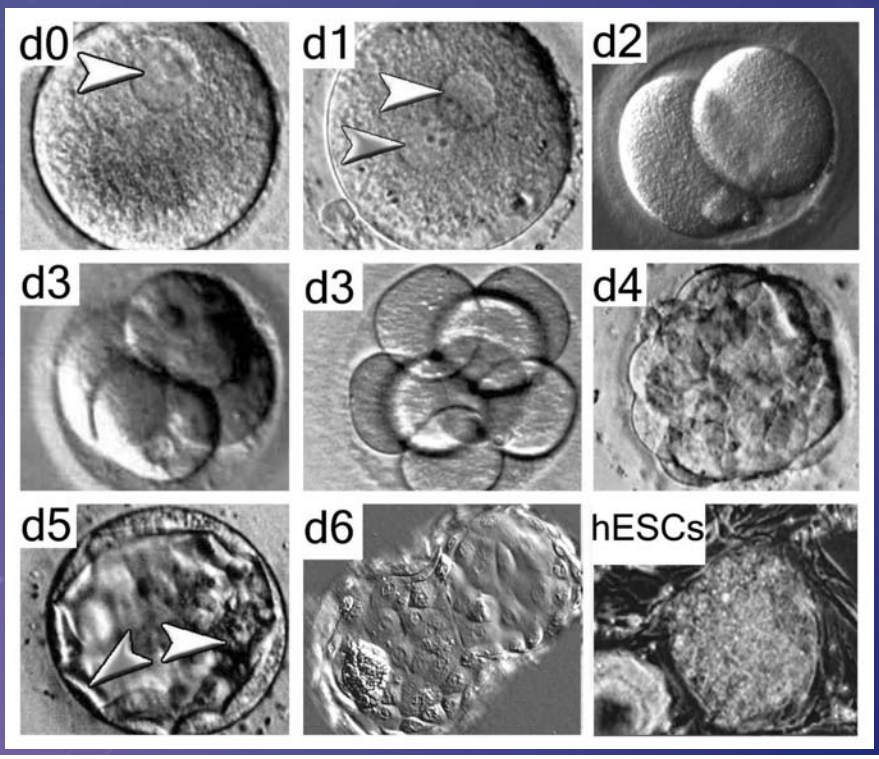


Stem Cell Division. *The stem cell divides asymmetrically, generating one cell that repeats the feat indefinitely, and one cell that continues to divide symmetrically, dividing each time into two equal daughter cells.*

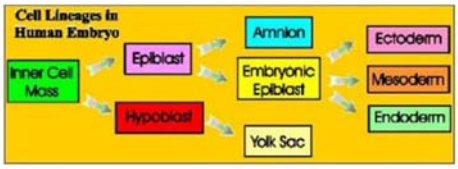
- Adult stem cells
- Fetal stem cells
- Embryonic stem cells

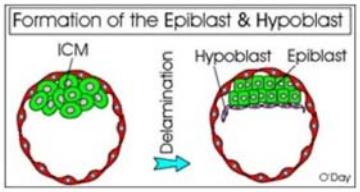
www.ISSCR.org

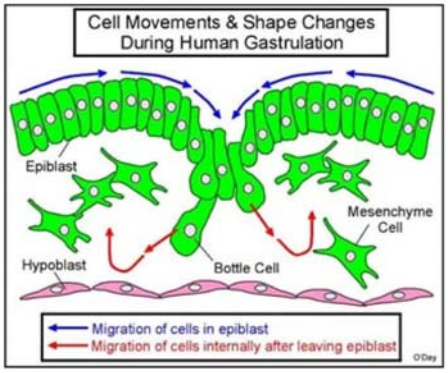
II. Human Embryonic Stem Cells: Development and Derivation

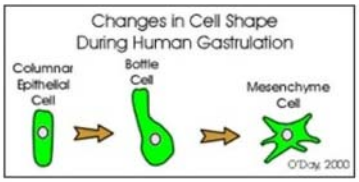


Human Development Continues: Developmental Programming









*Not all of these morphogenetic movements have been detailed in humans but they all have been shown to occur in other animals.

From: http://www.erin.utoronto.ca/~w3bio380

Human ES Cell (hESC) Lines

- NIH Embryonic Stem Cell Registry
- Cell lines from Cellartis AB, Goteborg, SWEDEN.
  - (2 NIH, 13 non-NIH-approved, 9 eligible for EU funding, 1 clonal line)
    Additional information: Stem Cells 22:367
- Cell lines from Harvard University, Harvard U.S.A.
  - 17 lines, non-NIH approved
- Cell lines from the Russian Academy of Science, Moscow, RUSSIA
  Additional information: ISSCR 2004, abstract #141
- Cell lines from the Reproductive Genetics Institute, Chicago, U.S.A.
  Additionlal information, **including cell lines with genetic defects**:
  ISSCR 2004, abstracts #115, 143, 370
- Cell lines from the University of Helsinki, FINLAND:
  - 4 lines non-NIH approved, non-eligible for EU funding
- Cell lines from the University of San Francisco, San Francisco, U.S.A.
  Additional information: lines **derived on human feeder** cells,
  ISSCR 2004, abstract #113
- Cell line from King's College London, UNITED KINGDOM
- Cell line from Royan Institute, Teheran, IRAN
  Additional information: Differentiation 75:224
- Cell line from University of Newcastle upon Tyne, UNITED KINGDOM
  Additional information: Stem Cells 22:790
- Cell lines from Brno, CZECH REPUBLIC
  - 7 lines non-NIH approved, additional information: Report (PDF)

Human Embryonic Stem Cell Differentiation

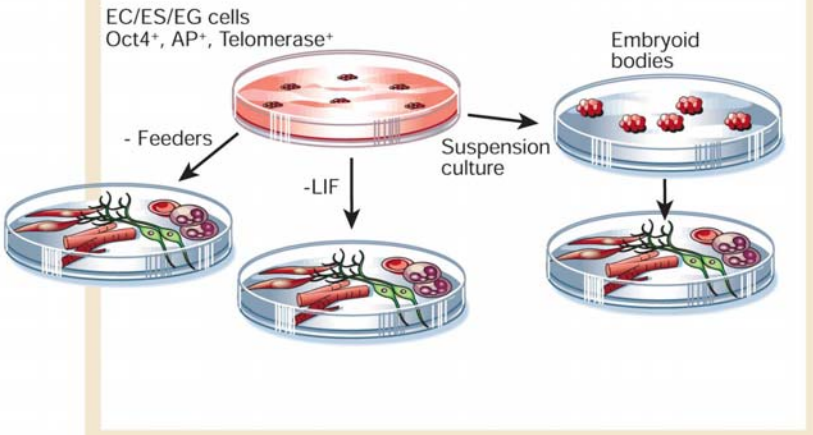
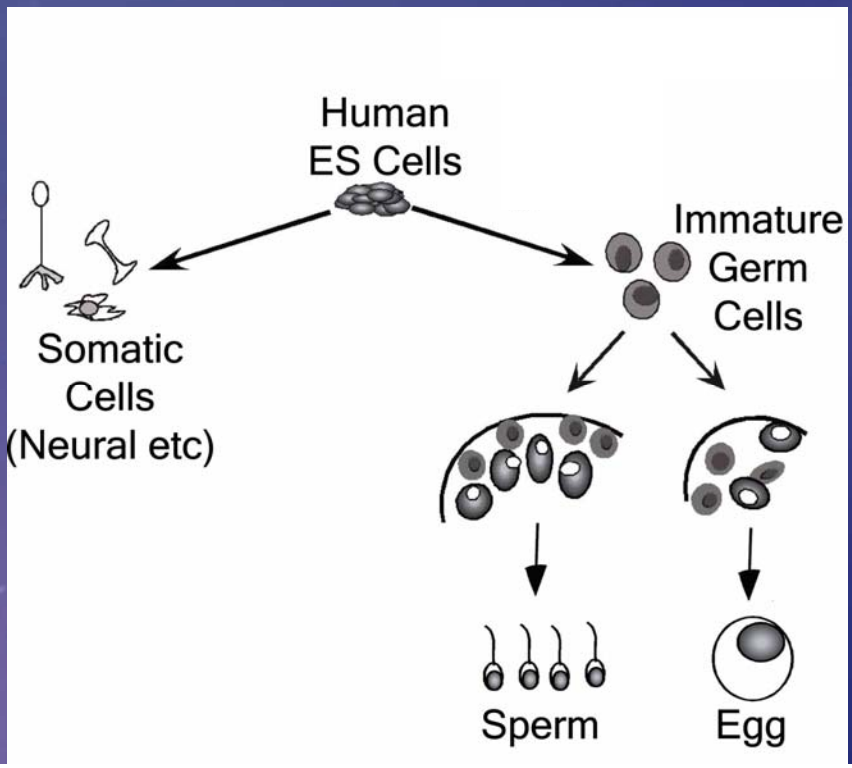


Figure 2 Differentiation of pluripotent stem cells into differentiated derivatives. Cultured EC, ES and EG cells can be induced to differentiate into a wide variety of differentiated derivatives in culture including pancreatic islet cells, blood cells, muscle cells and nerve cells. Differentiation can be induced by withdrawal of leukaemia inhibitory factor (LIF), separation of stem cells from feeder cells, or by growth of stem cell colonies in suspension culture to form embryoid bodies, which upon dissociation can be plated to yield differentiating cells.

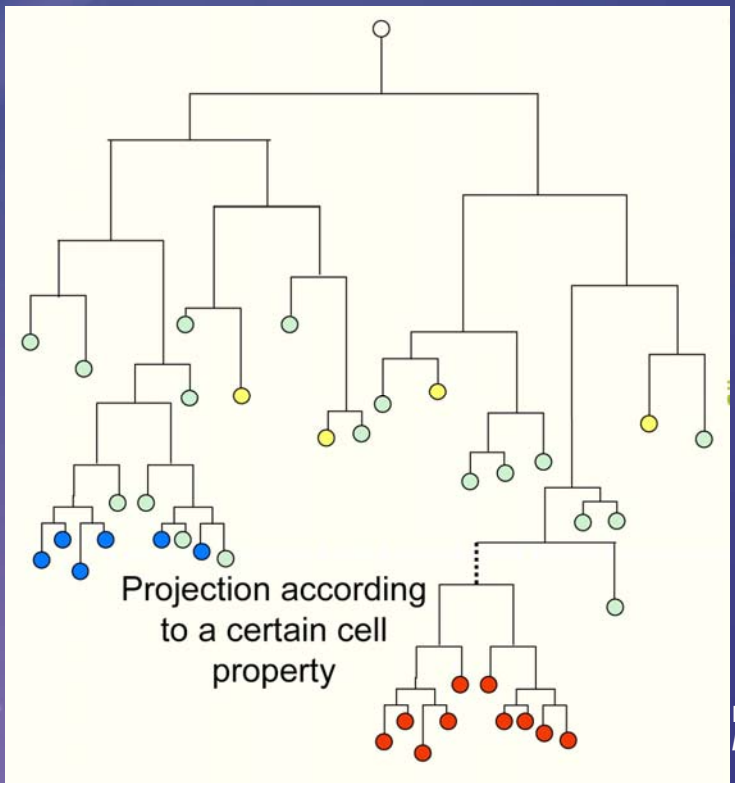
Donovan and Gearhart, 2001

hESC Lines Can Form All Cells of the Body: Somatic and Germ Cells



R. Reijo Pera

Human Development: A Series of Cell Decisions and Reduction of Potential



From: http://www.cosbi.eu/slides/Adam%20Wasserstrom.pdf

III. Alternatives: Reprogramming

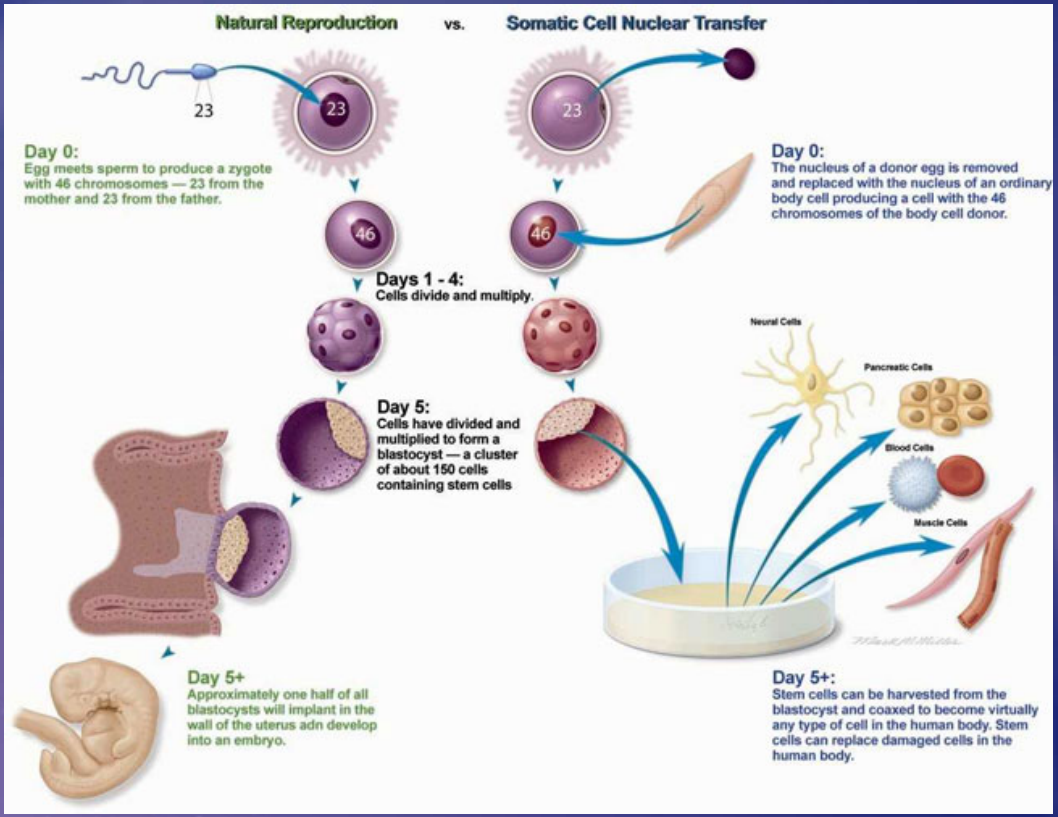
Programming:
The setting of cell fate during development. A skin cell divides to form a skin cell, a muscle cell divides to form a second muscle cell.

Reprogramming:
The erasure of established cell programs and re-establishment (re-setting or returning) to an embryonic cell.

A number of variations to redirect the programs of adult cells to an embryonic fate:

1. **Somatic cell nuclear transfer**
2. **Directed reprogramming with genetic factors**

SCNT



From http://www.kcchamber.com/

Directed Reprogramming

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,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]

[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2]CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
[3]Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA
[4]Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto 606-8507, Japan
*Correspondence: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2007.11.019

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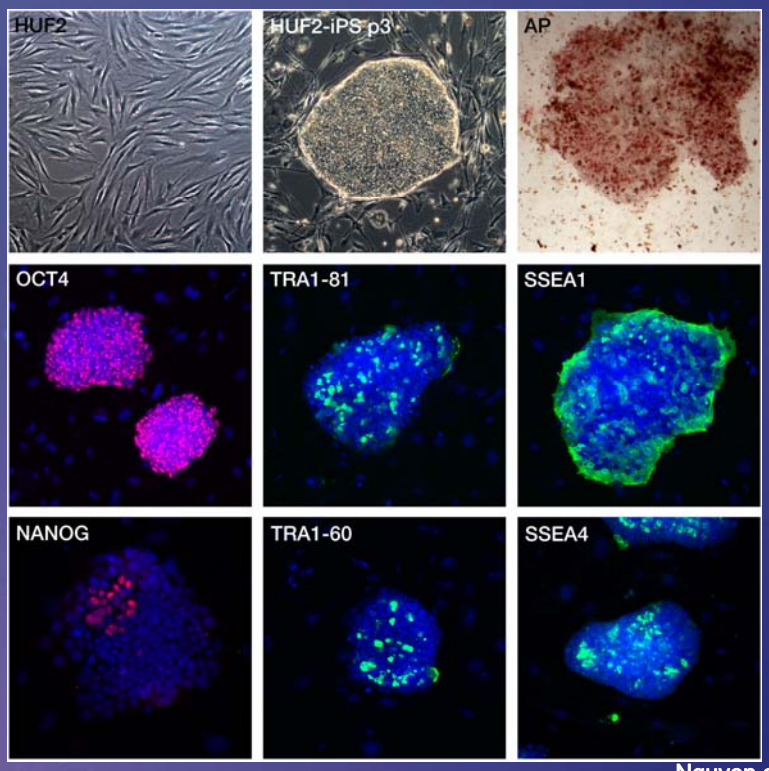
Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

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. Thomson[1,2,5]*

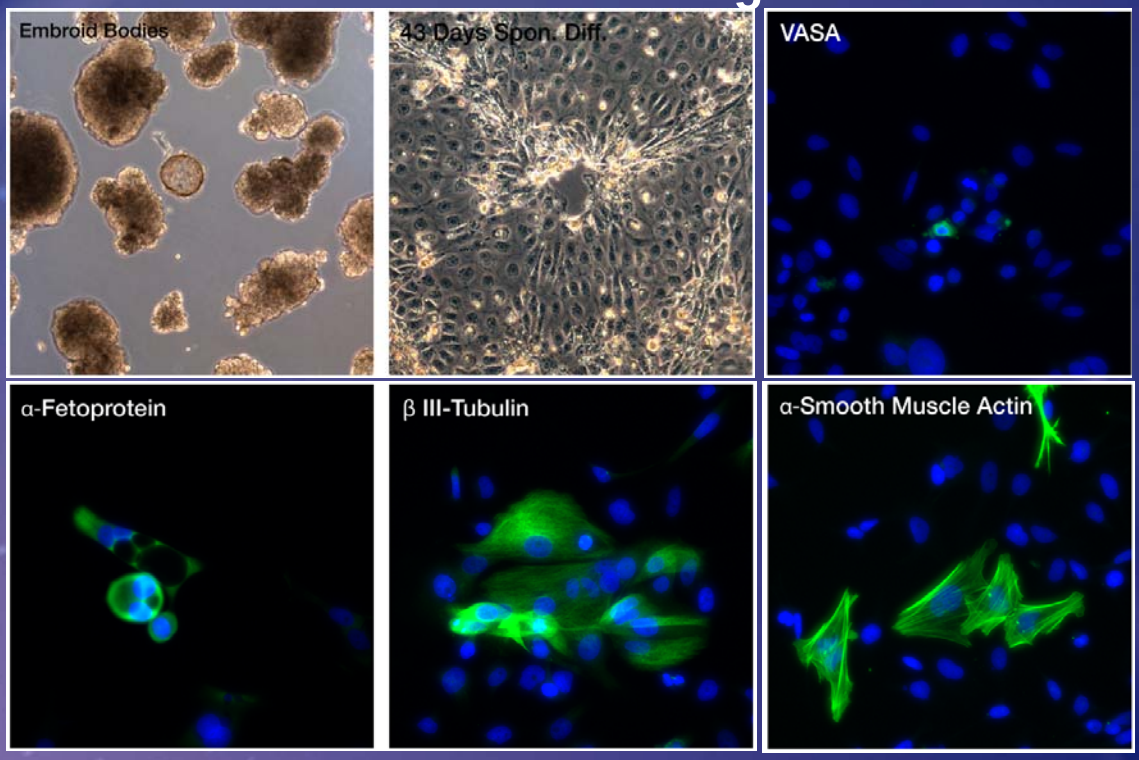
[1]Genome Center of Wisconsin, Madison, WI 53706–1580, USA. [2]Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, WI 53715–1299, USA. [3]WiCell Research Institute, Madison, WI 53707–7365, USA. [4]Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, WI 53706, USA. [5]Department of Anatomy, University of Wisconsin-Madison, Madison, WI 53706–1509, USA.

iPSCs Express Pluripotency Markers (HUF2-iPSCs from Adult Parkinson's Patient)



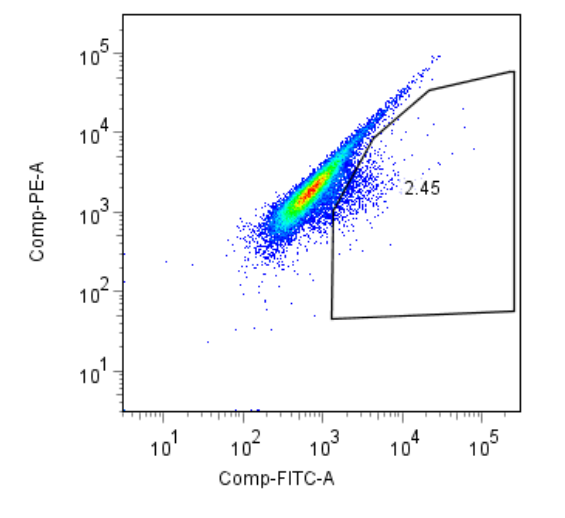
Nguyen et al, unpublished

iPSCs Differentiate to Both Somatic and Germ Cell Lineages

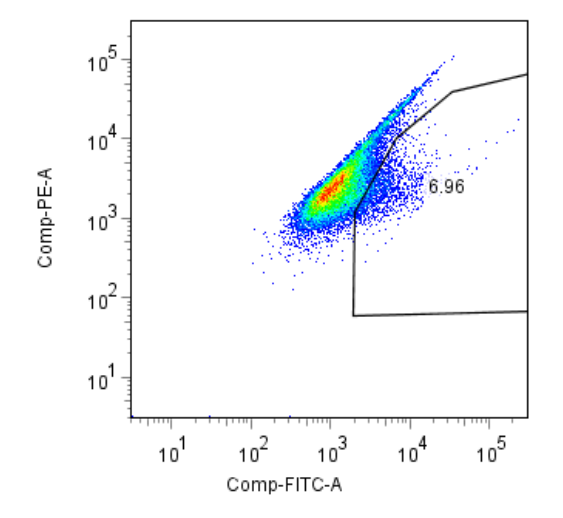


Nguyen et al, unpublished

Comparable Quantitative Differentiation of hESCs and iPSCs to the Germ Cell Lineage



HSF1+pLVGV
After 7 day BMPs diff.
2.45%VASA:GFP+



Huf2iPs+pLVGV
After 7 day BMPs diff.
6.96% VASA:GFP+

Panula et al, unpublished

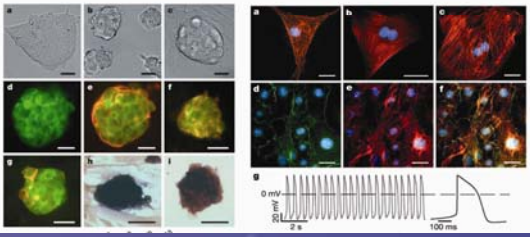
Cell Explantation and "ESC-Like Cells"

Vol 440|27 April 2006|doi:10.1038/nature04697 nature

LETTERS

Pluripotency of spermatogonial stem cells from adult mouse testis

Kaomei Guan[1]*, Karim Nayernia[2]*, Lars S. Maier[1], Stefan Wagner[1], Ralf Dressel[3], Jae Ho Lee[2], Jessica Nolte[2], Frieder Wolf[1], Manyu Li[2], Wolfgang Engel[2] & Gerd Hasenfuss[1]



Isolation and Characterization of Pluripotent Human Spermatogonial Stem Cell-Derived Cells

Nina Kossack,[a,e] Juanito Meneses,[b] Shai Shefi,[c,d] Ha Nam Nguyen,[a] Shawn Chavez,[a] Cory Nicholas,[a] Joerg Gromoll,[c] Paul J Turek[c]*, Renee A Reijo-Pera[a]*

[a]Institute for Stem Cell Biology and Regenerative Medicine; Department of Obstetrics and Gynecology; Stanford University School of Medicine, Palo Alto, CA 94304; [b]Center for Reproductive Sciences and [c]Department of Urology; University of California San Francisco, San Francisco, CA 94043; [d]Sheba Medical Center; Tel Hashomer 52621, Israel; [e]Center of Reproductive Medicine and Andrology, University of Muenster, Domagkstrasse 11, D-48129 Muenster, Germany

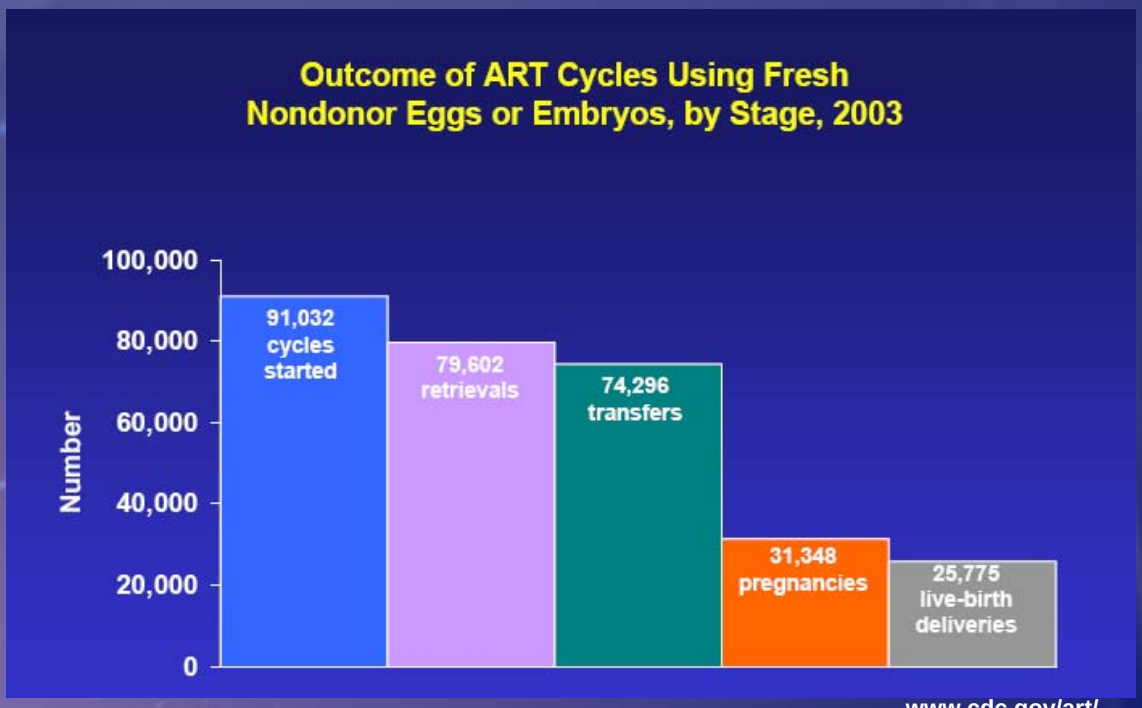
Key words. Human embryonic stem cells • Germline stem cells • Adult stem cells • Spermatogonia • Testis biopsy

Down

IV. Stem Cells and Health

Reproductive/Fetal Health
Somatic Health
Cancer

Lack of Knowledge of Human Development Impacts Reproductive Health and Fetal Outcome



Gene Expression During Reprogramming in the Human Oocyte – Embryo Transition

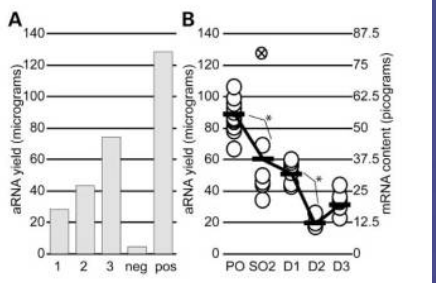
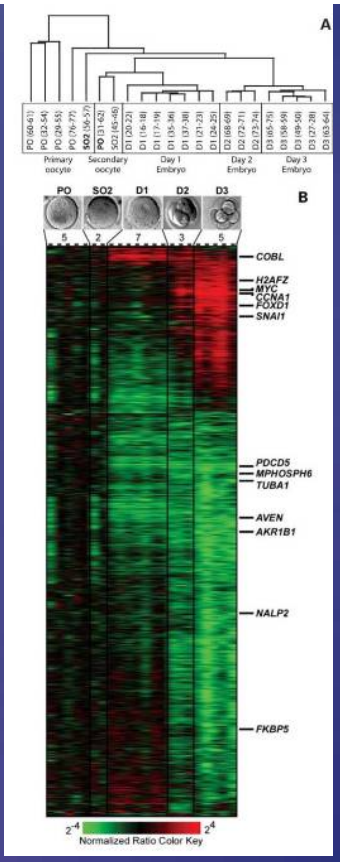


Figure 3. Determination of mRNA content of human oocytes and embryos. In (**A**) linearity of amplification yields versus starting material was demonstrated by amplification of RNA from different numbers of embryos ($N = 1$, 2 and 3 as shown on the x-axis). The embryos all contained six cells, except one of the embryos in the group of two contained seven cells. Negative control (neg) contained carrier DNA only and the positive control (pos) contained 2.5 ng of total RNA. In (**B**) on the y-axis, the average yield of aRNA (left) or mRNA content (right) was plotted as a function of stage of development listed on the x-axis. There were significant differences among the groups by ANOVA testing, and between the marked (*) groups by multiple comparison t-tests using the Bonferroni correction. RNA was amplified from primary oocytes (PO), secondary oocytes metaphase II (SO2), and embryos on day 1 (D1), day 2 (D2), and day 3 (D3) of development. The sample marked with an 'x' was a significant outlier as described in the text.

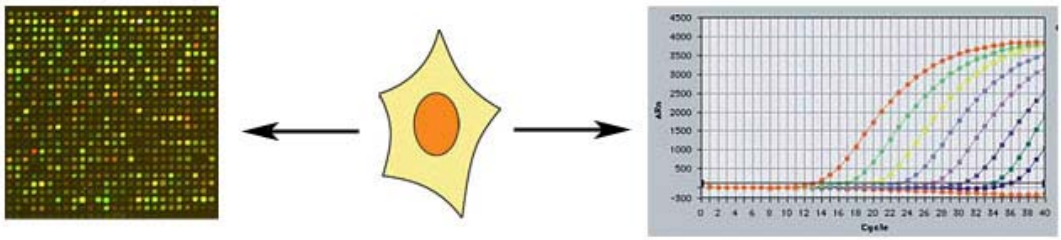


1896 genes are up- or down-regulated

Underlying clock: Cell division or time?
Equivalency/potency of cells?

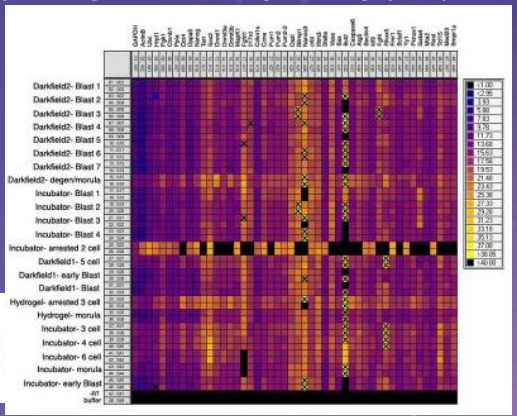
Dobson et al., 2004

Gene Expression in Single Blastomeres

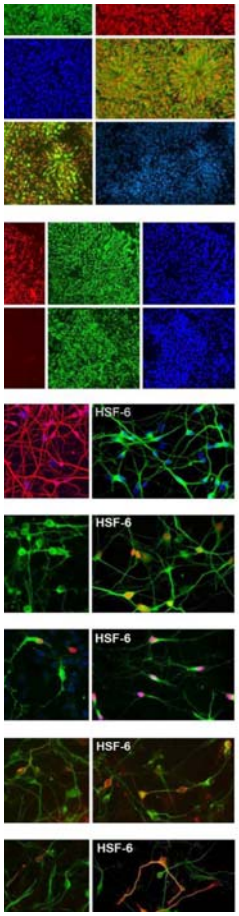


Global RNA Amplification using RiboAmp Ultra Kit from MDS Analytical Technologies

Gene-specific RNA Amplification followed by Fluidigm Biomark high throughput qPCR



Wong et al., unpublished



Embryonic Stem Cells and Somatic Health

Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts

Michael
Sarah K
Chris O
Chun Yi

Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive

Neuroscience 145 (2007) 1348–1358

re Publishing Group http://www.nature.com/naturebiotechnology

HUMAN EMBRYONIC STEM CELLS: LONG TERM STABILITY, ABSENCE OF SENESCENCE AND A POTENTIAL CELL SOURCE FOR NEURAL REPLACEMENT

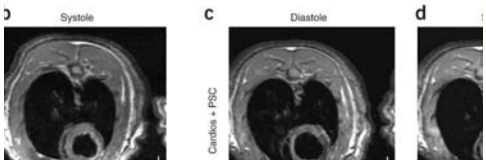
X. ZENG[a*] AND M. S. RAO[b,c]

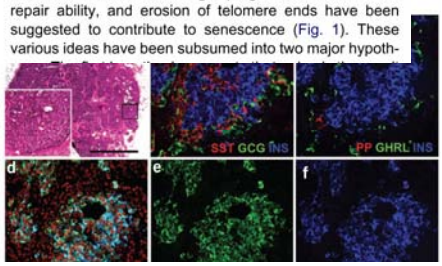
[a]*Buck Institute for Age Research, 8001 Redwood Boulevard, Novato, CA 94945, USA*

[b]*Neuroscience Faculty, Johns Hopkins School of Medicine, Baltimore, MD, USA*

[c]*Invitrogen Corp, Carlsbad, CA, USA*

senescence. In this state, they remain viable but are unable to divide further (Hayflick, 1976) (Fig. 1). Loss of genomic and mitochondrial genomic integrity, epigenetic alternation, oxidative damage, progressive loss of DNA repair ability, and erosion of telomere ends have been suggested to contribute to senescence (Fig. 1). These various ideas have been subsumed into two major hypoth-





Cancer Stem Cells

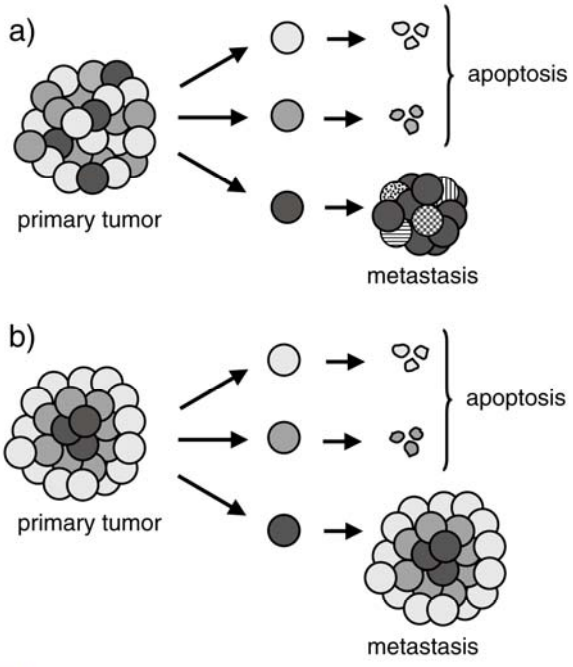


Figure 2

Impact of the cancer stem cell (CSC) model on the origin and biology of metastases. (*a*) According to standard cancer models, tumors are composed of heterogeneous mixtures of independent subclones, originated by divergent genetic mutations; different subclones are endowed with different functional properties, and only selected clones (*dark grey cells*) can migrate and form metastases. The metastasis is predicted to be a homogeneous monoclonal expansion of an individual subclone, which in turn can accumulate further mutations (*striped and variously patterned cells*) and diverge even further from the

Cancer Stem Cells: Models and Concepts

Piero Dalerba, Robert W. Cho, and Michael F. Clarke

Stanford Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, California 94304; email: pdalerba@stanford.edu, robcho@stanford.edu, mfclarke@stanford.edu

From: Dalerba et al, 2007

Stem Cells and Health Applications

Near-Term:
Reproductive Health and Fetal Outcomes

Intermediate-Term:
Improvements in Understanding/Diagnosis of Common and Rare Disease
Improvements in Cancer Treatment

Longer-Term:
Cell Replacement Therapies for Common Disorders from Heart Disease and Diabetes to Neural Degenerative Disorders

Overall Summary

Embryo development encompasses reprogramming and programming

- cell signaling
- epigenetic modification
- destruction of previous cell programs
- transcriptional activation
- translational and post-translational regulation

Differentiation is characterized by commitment of cells to fates and a reduction in pluripotency, with the exception of the germ line. hESC and iPSC differentiation of early germ cells is efficient, in many labs. Maturation requires optimization but is possible

Reprogramming: May recapitulate early developmental programs or be accomplished by convergence of diverse pathways. Appears to be complete in human somatic and germ cells

The "length of the trip:" Male germline stem cells susceptible to reprogramming relative to some other cell types

Major challenges

1) Directing cell decisions
   (optimized cell surfaces, molecular signals, cell interactions)

2) Analysis of single cells
   (gene expression, protein expression, epigenetic status, cell cycle length, morphology)

3) Diagnostics of fate
   (progenitor differentiation, tumorigenesis)

……. the end of all
our exploring will be to arrive where
we started and know the place
for the first time.

TS Eliot

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Martha Flores
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Kehkooi Kee
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Kevin Loewke
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Nam Nyguen
Sarita Panula
Sonya Schuh-Huerta
Henrike Siemen
Connie Wong

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March of Dimes
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Valerie Baker
Aaron Hsueh
Ruth Lathi
Lynn Westphal
Mylene Yao