SPERMATOGENESIS FROM STEM CELLS

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Disclosure

• Nothing to disclose
Hochedlinger, Development 2009 (adapted from Waddington, 1957)
Pluripotent Stem Cells can be obtained from cells located in the inner cell mass of blastocysts, early embryos and isolated blastomeres (hESC) and from nuclear reprogramming (Somatic Cell Nuclear Transfer - SCNT and induced Pluripotent Stem Cells - iPS)

Pluripotent stem cells: Self renewal/Differentiation

Asymmetric division  Self renewal  Differentiation
Somatic cell

Oct4
Klf4
Sox2

cMyc

Integrative delivery systems:
Retrovirus/lentivirus/linear DNA/PiggyBac transposon

Non integrative delivery systems:
Adenovirus/Sendai viral vectors/episomal vectors/synthetic mRNA/proteins

Blastocyst

Inner Cell Mass

Fertilization
Embryo
Activation
Parthenote

Ectoderm
Brain, Skin

Mesoderm
Muscle, Blood, Bone, Cartilage

Endoderm
Lung, Gut, Liver

Germline
Sperm, Egg

Macular Degeneration
Parkinson’s Disease
ALS

Heart Failure
Anemia, Leukemia
Arthritis, Bone Fracture

COPD
Diabetes
Cirrhosis

INDUCED PLURIPOTENT STEM CELLS

Somatic cell

INDBRYONIC STEM CELLS

EMBRYONIC STEM CELLS
Derivation of pluripotent stem cells from cultured human primordial germ cells

(alkaline phosphatase/embryoid body/embryonic stem cell/embryonic germ cell)

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Contributed by John W. Littlefield, September 29, 1998

REPORTS

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

www.sciencemag.org  SCIENCE  VOL 282  6 NOVEMBER 1998  1145
- 4 NT hESC were obtained with optimized SCNT approaches.
- Key factors for the success of SCNT:
  - Oocyte quality
  - Adequate exit from meiosis
  - Oocyte activation by electroporation
  - Improved embryo development with caffeine
- NT hESC displayed normal diploid karyotypes and inherited their nuclear genome from parental somatic cells
- Gene expression and differentiation profiles are similar to embryo derived hESC.
Human Somatic Cell Nuclear Transfer Using Adult Cells

Application of a recently developed methodology for the generation of human ESCs via SCNT using dermal fibroblasts from 35- and 75-year-old males.
Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors.
Takahashi K, Yamanaka S.
Cell. 2006.

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 Shinva Yamanaka1,2,3,4,*
Cell, 2007

Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells
Junying Yu,1,2* Maxim A. Vodyanik,2 Kim Smuga-Otto,3,2 Jessica Antosiewicz-Bourget,1,2 Jennifer L. Frane,2 Shulan Tian,3 Jeff Nie,3 Gudrun A. Jonsdottir,3 Victor Ruotti,3 Ron Stewart,3 Igor I. Slukvin,2,4 James A. Thomson1,2,3,*
Science, 2007
Induced pluripotent stem cells (iPS) generation

- The first iPS cell line generated with 24 factors. (Takahashi K & Yamanaka S. Cell 2006)

- The Classical 4 factors cocktail
  
  Oct4/3, Sox2, c- myc & KIF4
  Oct4/3, Sox2, Lin28 & Nanog

- Stable Karyotype
- Methylation of Nanog/Oct4 promoters
- Transgene expression silencing
- Expression of endogenous pluripotent associated markers
- In vitro/In vivo differentiation
- Chimera contribution*
Different cell types have been obtained

- Cardiomyocytes
- Neuronal cells
- Hematopoietic cells
- Pancreatic cells
- Hepatocytes
- ......
- Gametes: oocytes and sperm
Why are we interested in the generation of germ cells *in vitro*?

- To study gametogenesis *in vitro*
- To study meiosis *in vitro*
- To check the capability of PSC to form germ cells *in vitro*

- PSC may constitute a future source of artificial gametes for research and potential future therapeutic applications
- This system may provide a useful model for molecular genetic studies of human germline formation.
Important points to obtain male functional gametes during germline differentiation from pluripotent stem cells

The sequence of in vivo events:

Spermatogenesis

H19 gene

VASA

0% methylated

Sertoli and Leydig cells

75% methylated

Spermatogonial stem cell

Mitotic divisions

Sertoli and Leydig cells

Growth factors and hormones

MEIOSIS

H2AX

γH2AX

75% methylated

Acrosin

100% methylated

Primary spermatocyte

Meiosis I

Early spermatid

Secondary spermatocyte

Meiosis II

Sperm (Sertoli cells provide nutrients)
Generation of germ cells *in vitro* from mouse pluripotent stem cells

- **Pluripotent stem cells used**: ES, EG and iPS cells.
- **Sex of cell lines**: XY and XX.
- **Transgenic reporters / overexpression genes used**: MVH, Stra8, Prm1 and Stella.
- **Differentiation method used**: EB formation and monolayer differentiation.
- **Culture conditions**: FBS, BMP4, N2B27, Activin A, bFGF, Retinoic Acid, Transferrin, Monothyoglycerol and Ascorbic Acid.
- **In vitro cells obtained**: Epiblast, PGCs, SSCs and male and female haploid-like cells.
- **Epigenetic status of imprinted genes**: variable
- **Functional assays**: Transplantation into sterile testis and ovaries. ICSI. Offspring evaluation.
MOUSE

- Giejsen et al. Nature 2004*
- Toyooka et al. PNAS 2003
- Nayernia et al. Developmental Cell 2006*
- Eguizabal et al. Differentiation 2009
- Hayashi et al. Cell 2011*
- Nakaki et al, Nature 2013*
- Cai et al, BBRC 2013
Culture conditions: Embryoid body differentiation
During embryoid body formation **pluripotency markers expression decreased** with some expression of **GC specific genes**
Upregulation of genes associated with male germ cell development
EB microenvironment is permissive for **male germ cell development and meiotic maturation**, even though highly inefficient
EB derived cells have a similar morphology to testis derived haploid cells. **Round spermatids?**
ICSI with haploid cells into recipient oocytes

\[ \text{ICSI with haploid cells into recipient oocytes} \]
Establishment of two cell lines (Spermatogonial Stem Cells-SSCs) with patterns of differentiation towards male germ cells derived from mESC (directed gene expression for premeiotic and haploid male germ cells)

- Motility of cells
- Formation of tail-like structures

- ICSI of in vitro-generated cells (haploid cells) into oocytes of wildtype females.
- Polar body extrusion, PN formation and normal features of embryo development.
- Transfer into the oviducts of pseudopregnant females
- 65 embryos transferred, 12 animals born.
- Larger or smaller offspring. Premature death
- Abnormal methylation patterns and phenotypic abnormalities
Reconstruction of the Mouse Germ Cell Specification Pathway in Culture by Pluripotent Stem Cells

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DOI 10.1016/j.cell.2011.06.052

- Generation of in vitro primordial germ cell-like cells (PGCLC) from mES and miPS through epiblast-like cells having normal global transcription profiles, epigenetic reprogramming, and cellular dynamics.
Colonization of the Donor Cells in the W/W testes: transplantation into seminiferous tubules. Teratoma formation + spermatogenesis (colonies/testis) assessment

- These PGCLCs express Integrin-β3 and SSEA1 markers of spermatogonia.
- PGCLC transplantation into the seminiferous tubules of W/Wv neonatal mice lacking endogenous germ cells produced abundant spermatozoa with normal morphology.
- Fertilisation by ICSI: 2 PN zygotes and normal 2c and blastocysts
- Healthy offspring with normal placenta and birthweight
- Normal methylation patterns of imprinted genes
- Male and female offspring from PGCLC derived spermatozoa are fully fertile
Direct EpiLCs and mESC into primordial germ cells –like cells (PGCLCs) by over-expressing 3 TFs: BLIMP1 (B), PRDM14 (P14) and TFAP2C (A).

More than 80% of the cells expressed BV (Blimp1-mVenus) and 30% of the cells expressed SC (stella-eCFP).

- Simultaneous overexpression of 3 TFs directs EpiLCs, but not ESC, into a PGC state.
- TFs showed faster kinetics of Blimp1 and Stella induction than the cytokines.
- TFs directly activate a PGC program.
- Transcription factor induced PGC like cells contribute to spermatogenesis.
- Normal offspring were obtained from TF-PGCLCs.
Generation of germ cells *in vitro* from human pluripotent stem cells

- **Pluripotent stem cells used**: ES and iPS cells.
- **Sex of cell lines**: XY and XX.
- **Transgenic reporters / overexpression genes used**: DAZ, DAZL, BOULE and VASA.
- **Differentiation method used**: EB formation and monolayer differentiation.
- **Culture conditions**: FBS, BMP4, -7, -8b, Activin A, bFGF, hLIF, Retinoic Acid, R115866, Nicotinamide, Transferrin, Insulin, Selenium, Monothyoglycerol and Ascorbic Acid.
- **In vitro cells obtained**: PGCs, Putative Sertoli, SSCs and male haploid-like cells
- **Epigenetic status of imprinted genes**: correct
HUMAN

- Bucay *et al.* Stem Cells 2008
- Tilgner *et al.* Stem Cells 2008*;* 2009
- Park *et al.* Stem Cells 2009*
- Kee *et al.* Nature 2010
- Panula S *et al.* HMG 2011*
- Eguizabal *et al.* Stem Cells 2011*
- Medrano *et al.* Stem Cells 2011
- Easley *et al.*, Cell Reports 2012*
Isolation of Primordial Germ Cells from Differentiating Human Embryonic Stem Cells

Katarzyna Tilgner\textsuperscript{1,2}, Stuart P. Atkinson\textsuperscript{1,2}, Anna Golebiewska\textsuperscript{1,2}, Miodrag Stojkovic\textsuperscript{3}, Majlinda Lako\textsuperscript{1,2} and Lyle Armstrong\textsuperscript{1,2}.

Derivation of Primordial Germ Cells from Human Embryonic and Induced Pluripotent Stem Cells Is Significantly Improved by Coculture with Human Fetal Gonadal Cells

Tae Suk Park\textsuperscript{a,b}, Zoran Galic\textsuperscript{c}, Anne E. Conway\textsuperscript{a,b}, Anne Lindgren\textsuperscript{a,b}, Benjamin J. van Handel\textsuperscript{a,b}, Matthias Magnusson\textsuperscript{a,b}, Laura Richter\textsuperscript{a,b}, Michael A. Teitell\textsuperscript{d,e,f,g,h}, Hanna K. A. Mikkola\textsuperscript{a,b,f,g,h}, William E. Lowry\textsuperscript{a,b,f,g,h}, Kathrin Plath\textsuperscript{e,f,g,h}, i Amanda T. Clark\textsuperscript{a,b,f,g,h}.
Human germ cell differentiation from fetal- and adult-derived induced pluripotent stem cells

Sarita Panula¹,4, Jose V. Medrano¹,2, Kehkool Kee¹, Rosita Bergström⁴, Ha Nam Nguyen¹, Blake Byers¹,2, Kitchener D. Wilson³, Joseph C. Wu³, Carlos Simon⁵, Outi Hovatta⁶ and Renee A. Reljo Pera¹

- iPSC can form in vitro meiotic and post-meiotic haploid cells over-expressing DAZ, DAZL and BOULE.
• Generation of a novel differentiating 2 step-protocol **without overexpressing any genes.**
Meiotic progression of germ-like cells at 9 weeks of culture

- Immunofluorescence of meiotic spreads at 9 weeks of differentiation. Detection of VASA, SCP3 and H2AX in hESC and hiPS lines
Haploid formation of germ like cells at 10 weeks culture

- DNA content analysis
- FISH analysis (X,Y, 18)
- Percentages of 1n, 2n , 4 n and aneuploid cells in all samples.

*iPS can form in vitro meiotic and haploid like-cells.*

The post-meiotic-like cells generated have features of human spermatids expressing Acrosin.
Differentiation of hESCs and iPSCs in **SSC conditions** yields significant percentages of **VASA + cells** (10 days)

- hPSCs differentiated in SSC culture conditions exhibit haploid features
Differentiation of hPSCs in SSC culture yields cells that express markers for spermatogonia, spermatocytes and spermatids.

hESCs and hiPSCs differentiate directly into advanced male germ cell lineages, including postmeiotic, spermatid-like cells in vitro without genetic manipulation.
• Reconstitution of the essential first steps of pluripotent stem cell-based spermatogenesis in vitro has been established (mouse and human)
• Abnormal methylation patterns and offspring are achieved when the process is completed in in vitro conditions
• Normal healthy offspring is obtained with normal methylation patterns of imprinted genes if gametogenesis is resumed in in vivo conditions