Pluripotent of Stem Cell Literatures

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Abstract: The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell". Stem Cell is the original of life. All cells come from stem cells. Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situation or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a typical and important topic of life science. This material collects some literatures on pluripotent of stem cell.
[Smith MH. **Pluripotent of Stem Cell Literatures.** *Stem Cell* 2012;3(1):33-70] (ISSN 1545-4570). http://www.sciencepub.net/stem. 4

Key words: stem cell; life; gene; DNA; protein; pluripotent

1. Introduction

Stem cells describe all of the cells that can give rise to the different cells found in tissues. Under a right condition, a stem cell can become all type of the cells in the body. Cell p**luripotency** means a stem cell having the potential to differentiate into any of the three germ layers: ectoderm (epidermal tissues and nervous system, etc), endoderm (interior stomach lining, gastrointestinal tract and lungs, etc) or mesoderm (muscle, bone, blood and urogenital, etc). Pluripotent stem cells can be differentiated to any fetal or adult cell type. However, the Pluripotent stem cells cannot develop into a fetal or adult organism alone because they are lack of the potential to contribute to extraembryonic tissue, such as the placenta. After the embryonic development stage is over, the stem cells no longer have this unlimited potential to develop into all cell types, the pluripotency is lost and they can only become certain types of cells.

Induced pluripotent stem cells (iPSCs) are genetically reprogrammed adult cells that exhibit a pluripotent stem cell-like state similar to embryonic stem cells.[1] While these artificially generated cells are not known to exist in the human body, they show qualities remarkably similar to those of embryonic stem cells (ESCs); thus, iPSCs are an invaluable resource for drug discovery, cell therapy, and basic research.

After an egg is fertilised by a sperm, a single cell comes out. This fertilised egg is totipotent which has the potential ability to create an entire organism. However, the totipotent cells change to pluripotent cells that lost the ability of totipotent – the pluripotent cannot differentiate to a entire body.

There are several key types of pluripotent stem cells: (1) Embryonic stem cells are isolated from the inner cell mass of the blastocyst. (2) Embryonic germ cells are taken from aborted foetuses and these pluripotent cells are derived from very early cells. (3) Embryonic carcinoma or cancer cells are isolated from a type of tumour that sometimes occurs in a foetus.

Yamanaka and colleagues first demonstrated that retrovirus-mediated delivery and expression of Oct4, Sox2, c-Myc and Klf4 is capable of inducing the pluripotent state in mouse fibroblasts, and they also reported the successful reprogramming of human somatic cells into induced pluripotent stem (iPS) cells using human versions of the same transcription factors delivered by retroviral vectors. The generation of patient-specific iPS cells circumvents an important roadblock to personalized regenerative medicine therapies by eliminating the potential for immune rejection of non-autologous transplanted cells. (Wu, Hamilton et al. 2009).

Mouse and human fibroblasts have been transformed into induced pluripotent stem (iPS) cells by retroviral transduction or plasmid transfection with four genes. Tumor formation has been found in offspring of mice generated from blastocysts made mosaic with iPS cells. The adenoviral vectors can reprogram human fibroblasts to pluripotent stem cells for use in individualized cell therapy without the risk for viral or oncogene incorporation (Zhou and Freed 2009).

Domesticated ungulate pluripotent embryonic stem (ES) cell lines would be useful for generating precise gene-modified animals. Many efforts have been made to establish domesticated ungulate pluripotent ES cells from early embryos without success. Wu, et al, reported that properties of porcine pluripotent stem cells that may facilitate the eventual establishment of porcine ES cells (Wu, Chen et al. 2009).

Pluripotent stem cells have the potential for treatment of many diseases. Pluripotent stem cells can evolve into specialised cells that ultimately can replace diseased cells and tissues. The positive uses of pluripotent stem cells are enormous but new research and ethical challenges must be taken into account before the public can reap the full benefits. For those who suffer from the many diseases that may be treated by pluripotent stem cells, additional knowledge and research will hopefully come sooner rather than later.

Human induced pluripotent stem (iPS) cells hold great promise for cardiovascular research and therapeutic applications, but the ability of human iPS cells to differentiate into functional cardiomyocytes has not yet been demonstrated (Zhang, Wilson et al. 2009). Reprogramming differentiated human cells to induced pluripotent stem (iPS) cells has applications in basic biology, drug development, and transplantation. Human iPS cell derivation previously required vectors that integrate into the genome, which can create mutations and limit the utility of the cells in both research and clinical applications (Yu, Hu et al. 2009). Human induced pluripotent stem (iPS) cells derived from somatic cells hold promise to develop novel patient-specific cell therapies and research models for inherited and acquired diseases (Ye, Zhan et al. 2009).

Invitrogen protocol examples (Invitrogen reagents are good as our experiences):

Materials

- Episomal iPSC Reprogramming Vectors (50 µL, 1 µg/µL) (Invitrogen, Cat. no. A14703)
- Dulbecco's Modified Eagle Medium (DMEM) with GlutaMAX™-I (High Glucose) (Invitrogen, Cat. no. 10569-010)
- KnockOut™ DMEM/F-12 (Invitrogen, Cat. no. 12660-012)
- Fetal Bovine Serum (FBS), ESC-Qualified, US Origin (Invitrogen, Cat. no. 16141-079)
- MEM Non-Essential Amino Acids Solution, 10 mM (Invitrogen, Cat. no. 11140-050)
- Basic Fibroblast Growth Factor (bFGF) (Invitrogen, Cat. no. PHG0264)
- HA-100 (ROCk inhibitor) (Santa Cruz, Cat. no. sc-203072)
- Bovine Albumin Fraction V Solution (BSA) (Invitrogen, Cat. no. 15260-037)
- Essential 8™ Medium (Prototype), consisting of DMEM/F-12 (HAM) 1:1 and Essential 8™ Supplement (50X) (Invitrogen, Cat. no. A14666SA)
- DMEM/F-12 with HEPES (Invitrogen, Cat. no. 11330-057)
- N-2 Supplement (100X) (Invitrogen, Cat. no. 17502-048)
- B-27® Supplement (50X) (Invitrogen, Cat. no. 17504-044)
- GlutaMAX™-I (100X) (Invitrogen, Cat. no. 35050-061)
- β-mercaptoethanol, 1000X (Invitrogen, Cat. no. 21985-023)
- PD0325901(MEK Inhibitor) (Stemgent, Cat. no. 04-0006)
- CHIR99021 (GSK3β inhibitor) (Stemgent, Cat. no. 04-0004)
- A-83-01 (TGF-β/Activin/Nodal receptor inhibitor) (Stemgent, Cat. no. 04-0014)
- hLIF (Human Leukemia Inhibitory Factor) (Invitrogen, Cat. no. PHC9461)
- Vitronectin, truncated recombinant human (VTN-N) (Invitrogen, Cat. no. A14701SA) or Geltrex® LDEV-Free hESC Qualified Reduced Growth Factor Basement Membrane Matrix (Invitrogen, Cat. no. A1413301)
- 0.05% Trypsin-EDTA (1X), Phenol Red (Invitrogen, Cat. no. 25300-054)
- UltraPure™ 0.5 M EDTA, pH 8.0 (Invitrogen, Cat. no. 15575-020)
- Dulbecco's PBS (DPBS) without Calcium and Magnesium (Invitrogen, Cat. no. 14190-144).
- Characterization reagents (surface marker staining):
  Mouse primary antibodies (one is required):
  - Mouse Anti-Tra1-60 Antibody (Invitrogen, Cat. no. 41-1000)
  - Mouse Anti-Tra1-81 Antibody (Invitrogen, Cat. no. 41-1100)
  - Mouse Anti-SSEA4 Antibody (Invitrogen, Cat. no. 41-4000)

  Alexa Fluor® secondary antibodies (one is required):
  - Alexa Fluor® 488 Goat Anti-Mouse IgG (H+L) Antibody (Invitrogen, Cat. no. A11029)
  - Alexa Fluor® 594 Goat Anti-Mouse IgG (H+L) Antibody (Invitrogen, Cat. no. A11032)
  - Alexa Fluor® 488 Goat Anti-Rabbit IgG (H+L) Antibody (Invitrogen, Cat. no. A11034)
  - Alexa Fluor® 594 Goat Anti-Rabbit IgG (H+L) Antibody (Invitrogen, Cat. no. A11037)
- Detection reagents (for detection of episomal vectors using PCR)
  - CellsDirect Resuspension & Lysis Buffers (Invitrogen, Cat. no. 11739-010)
  - AccuPrime Taq High Fidelity (Invitrogen, Cat. no. 12346-094)
  - Forward and Reverse primers for PCR (primer sequences are given in the PCR protocol)
- Electroporation instrument (e.g., Neon® Transfection System, Cat. no. MPK5000)
- 37°C water bath
- Appropriate tissue culture plates and supplies

Protocol

A typical reprogramming schedule using the Episomal iPSC Reprogramming Vectors is shown below:

Day –4 to –2:		Plate human fibroblasts into a T75 flask in Fibroblast Medium so that they are 75–90% confluent on the day of transfection (Day 0).
Day 0:		Transfect the cells using the Neon® Transfection System. Plate transfected cells onto vitronectin-coated culture dishes and incubate them overnight in Supplemented Fibroblast Medium.
Day 1 to 14:		Change the medium to N2B27 Medium supplemented with CHALP molecule cocktail and bFGF; replace the spent medium every other day.
Day 15:		Change the medium to Essential 8™ Medium and monitor the culture vessels for the emergence of iPSC colonies.
Day 21:		Pick and transfer undifferentiated iPSCs onto fresh vitronectin-coated culture dishes for expansion.

Preparing Media and Materials

10 µg/mL bFGF Solution (1000 µL)

1. To prepare 1 mL of 10 µg/mL bFGF solution, aseptically mix the following components:

Component	Volume
bFGF	10 µg
DPBS without Calcium and Magnesium	980 µL
BSA	10 µL

2. Aliquot and store at –20°C (or for –20°C up to 6 months – 12 months.

Fibroblast Medium (for 100 mL of complete medium)

1. To prepare 100 mL of Fibroblast Medium, aseptically mix the following components:

Component	Volume
DMEM	89 mL
FBS, ESC-Qualified	10 mL
MEM Non-Essential Amino Acids Solution, 10 mM	1 mL

2. Fibroblast Medium can be stored at 4°C for up to 2 weeks.

Supplemented Fibroblast Medium (for 100 mL of complete medium)

Note: You will need 30 mL of Supplemented Fibroblast Medium per transfection.

1. To prepare 100 mL of Supplemented Fibroblast Medium, add the following components to Fibroblast Medium freshly, just prior to use:HA-100 (ROCk inhibitor) varies (final concentration = 10 µgM)
2. bFGF (10 µg/mL) 40 ìL (final concentration = 4 ng/mL)
3. Supplemented Fibroblast Medium must be used once HA-100 and bFGF are added to the medium.

Essential 8™ Medium (500 mL of complete medium)

1. Thaw Essential 8™ Supplement (50X) at 4°C overnight. Do not thaw the medium at 37°C.
2. To prepare 500 mL of complete Essential 8™ Medium, aseptically mix the following components:
3. DMEM/F-12 (HAM) 1:1 490 mL
4. Essential 8™ Supplement (50X) 10 mL
5. Complete Essential 8™ Medium can be stored at 4°C for up to 2 weeks. *Note:* Before use, warm complete medium required for that day at room temperature until it is no longer cool to the touch. Do not warm the medium at 37°C.

N2B27 Medium (250 mL of complete medium)

1. To prepare 250 mL of N2B27 Medium, aseptically mix the following components:

Component	Volume
DMEM/F-12 with HEPES	238.75 mL
N-2 Supplement (100X)	2.5 mL
B-27® Supplement (50X)	5.0 mL
MEM Non-Essential Amino Acids Solution, 10 mM	2.5 mL
GlutaMAX™-I (100X)	1.25 mL
β-mercaptoethanol, 1000X	454.5 µL

2. To supplement N2B27 Medium with CHALP molecule cocktail and bFGF), add the following components to the indicated concentration. These must be added freshly, just prior to use.

Component	Volume
PD0325901 (MEK inhibitor)	0.5 µM
CHIR99021 (GSK3â inhibitor)	3 µM
A-83-01 (TGF-β/Activin/Nodal receptor inhibitor)	0.5 µM
hLIF (Human Leukemia Inhibitory Factor)	10 ng/mL
HA-100 (ROCk inhibitor)	10 µM
bFGF (10 µg/mL)	100 ng/mL

3. N2B27 Medium (without CHALP molecules and bFGF) can be stored at 4°C for up to 1 week.

0.5 mM EDTA in DPBS (50 mL)

1. To prepare 50 mL of 0.5 mM EDTA in DPBS, aseptically mix the following components in a 50-mL conical tube in a biological safety cabinet:
2. DPBS without Calcium and Magnesium 50 mL
3. 0.5 M EDTA 50 ìL
4. Filter sterilize the solution. The solution can be stored at room temperature for up to 6 months.

Coating Culture Vessels with Vitronectin (VTN-N)

1. Remove a 1-mL vial of vitronectin from –70°C storage and thaw at 4°C overnight.
2. Prepare working aliquots by dispensing 60 ìL of vitronectin into polypropylene tubes. The working aliquots can be frozen at –70°C or used immediately.
3. Prior to coating culture vessels, calculate the working concentration of vitronectin using the formula below and dilute the stock appropriately.
4. The optimal working concentration of vitronectin is cell line dependent.
5. To coat the wells of a 6-well plate, remove a 60-ìL aliquot of vitronectin from –70°C storage and thaw at room temperature. You will need one 60-ìL aliquot per 6-well plate.
6. Add 60 ìL of thawed vitronectin into a 15-mL conical tube containing 6 mL of sterile DPBS without Calcium and Magnesium at room temperature. Gently resuspend by pipetting the vitronectin dilution up and down. Note: This results in a working concentration of 5 ìg/mL (i.e., a 1:100 dilution).
7. Aliquot 1 mL of diluted vitronectin solution to each well of a 6-well plate (refer to Table 1 for recommended volumes for other culture vessels).
8. Note: When used to coat a 6-well plate (10 cm2/well) at 1 mL/well, the final concentration will be 0.5 ìg/cm2.
9. Incubate at room temperature for 1 hour.
10. Note: Dishes can now be used or stored at 4°C wrapped in laboratory film for up to a week. Do not allow the vessel to dry. Prior to use, pre-warm the culture vessel to room temperature for at least 1 hour.
11. Aspirate the diluted vitronectin solution from the culture vessel and discard. It is not necessary to rinse off the culture vessel after removal of vitronectin. Cells can be passaged directly onto the vitronectin-coated culture dish. Note: Geltrex® LDEV-Free hESC-Qualified Reduced Growth Factor Basement Membrane Matrix may be substituted for vitronectin (see the Appendix).

Table 1. Volume of Diluted Vitronectin Required

Culture Vessel	Surface Area (cm^2)	Volume of Diluted Substrate (mL)
6-well plate	10 cm^2/well	1 mL/well
12-well plate	4 cm^2/well	0.4 mL/well
24-well plate	2 cm^2/well	0.2 mL/well
35-mm dish	10 cm^2	1 mL
60-mm dish	20 cm^2	2 mL
100-mm dish	60 cm^2	6 mL

Reprogramming Fibroblasts

The following protocol has been optimized for human neonatal foreskin fibroblast cells (strain BJ; ATCC no. CRL2522). We recommend that you optimize the protocol for your cell type.

Day –4 to –2: Seed Cells

1. Two to four days before transfection, plate human fibroblast cells in Fibroblast Medium into a T75 flask. Cells should be approximately 75–90% confluent on the day of transfection (Day 0).
2. Note: Growth rate is dependent on the cell line and culture conditions. Depending on the seeding density and culture conditions, the cells may take up to 5 days to reach 75–90% confluency.
3. *Note:* Since overconfluency results in decreased transfection efficiency, we recommend replating your cells to achieve 75–90% confluency if your cells have become overconfluent during culturing.

Day 0: Prepare the cells for transfection

Note: Gentle handling of the cells prior to transfection is essential for the success of the transfection procedure.

4. Add 6 mL of Supplemented Fibroblast Medium to a 15-mL conical tube for each transfection (1 tube per transfection). Incubate tube at 37°C until needed (see step 23).
5. Aspirate medium from vitronectin-coated plates and replace with 12 mL of fresh Supplemented Fibroblast Medium per plate. Place the coated plates at 37°C until ready for use.
6. *Note:* You will need two 100-mm vitronectin-coated dishes for each transfection.
7. Aspirate the spent medium from the fibroblasts in T75 flasks.
8. Wash the cells in DPBS without Calcium and Magnesium.
9. Add 2 mL of 0.05% Trypsin/EDTA to each flask.
10. Incubate the flasks at 37°C for approximately 4 minutes.
11. Add 6 mL Supplemented Fibroblast Medium to each flask. Tap the plate against your hand to ensure cells have been dislodged from the plate, and carefully transfer cells into an empty 15-mL conical tube.
12. *Note:* Each T75 flask provides plenty of cells for transfection, so any residual cells still clinging to the flask after Trypsin/EDTA treatment may be left behind.
13. Remove a 20-µL sample to perform a viable cell count and calculate the number of transfection to be performed. You will need 1 × 106 cells for one transfection.
14. Number of transfections = Number of viable cells/(1×10^6)
15. Transfer enough cells for up to three transfections (i.e., 1×10^6 to 3×10^6 cells) into a new 15-mL conical tube.
16. Bring the volume to 10 mL in the new tube with Supplemented Fibroblast Medium and centrifuge cells at 1,000 rpm for 5 minutes at room temperature.
17. Carefully aspirate most of the supernatant, using a glass Pasteur pipette, leaving approximately 100–200 µL behind. Remove the remaining medium with a 200-µL pipette.

Day 0: Transfection

18. Resuspend the cell pellet in Resuspension Buffer R (included with Neon® Transfection kits) at a final concentration of 1.0×10^6 cells/0.1 mL.
19. Transfer the cells (100 µL per transfection reaction) to a sterile 1.5-mL microcentrifuge tube.
20. Turn on the Neon® unit and enter the electroporation parameters in the Input window (see Table 2).
21. **Table 2** Electroporation Parameters for Neon® Transfection System

Pulse Voltage	Pulse Width	Pulse Number	Cell Density	Tip Type
1650 V	10 ms	3	1×10^6 cells/0.1 mL	100 µL

22. Fill the Neon® Tube with 3 mL Electrolytic Buffer (use Buffer E2 for the 100 µL Neon® Tip).
23. Insert the Neon® Tube into the Neon® Pipette Station until you hear a click.
24. Transfer 8.5 µL Episomal Reprogramming Vectors to the tube containing cells and mix gently.
25. Insert a Neon® Tip into the Neon® Pipette.
26. Press the push-button on the Neon® Pipette to the first stop and immerse the Neon® Tip into the cell-DNA mixture. Slowly release the push-button on the pipette to aspirate the cell-DNA mixture into the Neon® Tip.
27. Note: Avoid air bubbles during pipetting to avoid arcing during electroporation. If you notice air bubbles in the tip, discard the sample and carefully aspirate fresh sample into the tip again without any air bubbles.
28. Insert the Neon® Pipette with the sample vertically into the Neon® Tube placed in the Neon® Pipette Station until you hear a click.

29. Ensure that you have entered the appropriate electroporation parameters and press Start on the Neon® touchscreen to deliver the electric pulse.
30. Note: After the electric pulse is delivered, the touchscreen displays "Complete" to indicate that electroporation is complete.
31. Remove the Neon® Pipette from the Neon® Pipette Station and immediately transfer the samples from the Neon® Tip into the 15-mL tube containing 6 mL of pre-warmed Supplemented Fibroblast Medium (prepared in step 2).
32. Mix the transfected cells by gentle inversion and pipette 3 mL into the 100-mm vitronectin-coated plate (two plates per transfection). Evenly distribute cells across plate. Discard the Neon® Tip into an appropriate biological hazardous waste container.
33. Repeat the process for any additional samples. Do not use Neon® tip more than twice.
34. Incubate the plates at 37°C in a humidified CO2 incubator overnight.

Day 1: Switch to Supplemented N2B27 Medium

35. Aspirate the spent Supplemented Fibroblast Medium from the plates using a Pasteur pipette.
36. Add 10 mL N2B27 Medium supplemented with CHALP molecule cocktail and bFGF (added freshly prior to use) to each 100-mm plate.
37. Replace the spent medium every other day, up to day 15 post-transfection.

Day 15: Switch to Essential 8™ Medium

38. Aspirate the spent medium and replace with Essential 8™ Medium. Resume medium changes every other day.
39. Observe the plates every other day under a microscope for the emergence of cell clumps indicative of transformed cells (see Figure 1). Within 15 to 21 days of transfection, the iPSC colonies will grow to an appropriate size for transfer.

Identifying iPSC Colonies

By Day 21 post-transduction, the cell colonies on the vitronectin-coated plates are large and compact, covering the majority of the surface area of the culture vessel. However, only a fraction of these colonies will consist of iPSCs, which exhibit a hESC-like morphology characterized by a flatter cobblestone-like appearance with individual cells clearly demarcated from each other in the colonies (see Figure 2). Therefore, we recommend that you perform live staining with Tra1-60 or Tra1-81 antibodies that recognize undifferentiated iPSCs.

Live Staining with Antibodies

One of the fastest and most reliable methods for selecting a reprogrammed colony is live staining with Tra1-60 or Tra1-81 antibodies that recognize undifferentiated iPSCs and enable the identification of reprogrammed cells from a variety of human cell types.

Note: Other methods of identifying iPSCs (such as alkaline phosphatase staining) are also acceptable.

1. Aspirate the medium from the reprogramming dish.
2. Wash the cells once with KnockOut™ DMEM/F-12.
3. Add the diluted primary antibody (Mouse Anti-Tra 1-60, Mouse Anti-Tra 1-81, or Mouse Anti-SSEA; see Materials Needed) to the cells (6 mL per 100-mm dish).
4. Incubate the primary antibody and the cells at 37°C for 60 minutes.
5. Remove the primary antibody solution from the dish.
6. *Note:* The primary antibody solution can be stored at 4°C for one week and re-used up to two times.
7. Wash the cells three times with KnockOut™ DMEM/F-12.
8. Add the diluted secondary antibody to the cells (6 mL per 100-mm dish).
9. Note: Any of the four Alexa Fluor® secondary antibodies listed in the Materials Needed section can be used.
10. Incubate the secondary antibody and the cells at 37°C for 60 minutes.
11. Remove the secondary antibody solution from the dish.
12. *Note:* The secondary antibody solution can be stored at 4°C for one week and re-used up to two times.
13. Wash cells three times with KnockOut™ DMEM/F-12. Add fresh KnockOut™ DMEM/F-12 to cover the surface of the cells (6 mL per 100-mm dish).
14. Visualize the cells under a standard fluorescent microscope and mark the successfully reprogrammed colonies for picking and expansion. Successful antibody staining can very specifically distinguish reprogrammed colonies from just plain transformed counterparts (see Figure 3), and can be detected for up to 24–36 hours. This is particularly useful because it helps identifying and tracking of candidate iPSC colonies before picking and the day after they are transferred into a new culture dish for expansion.

Detecting Episomal Vectors by PCR

Preparing iPSCs for PCR

Note: Endpoint PCR is the suggested method for verifying the loss of the episomal vectors over time.

1. Aspirate the medium from the dish containing iPSCs with a Pasteur pipette, and rinse the dish twice with Dulbecco's PBS (DPBS) without Calcium and Magnesium. Refer to Table 3 for the recommended volumes.

Table 3 Volume of Reagents Required

Culture Vessel	Approximate Surface Area (cm^2)	DPBS (mL)	0.5 mM EDTA in DPBS (mL)	Complete Essential 8™ Medium (mL)
6-well plate	10 cm^2/well	2 mL/well	1 mL/well	2 mL/well
12-well plate	4 cm^2/well	1 mL/well	0.4 mL/well	1 mL/well
24-well plate	2 cm^2/well	0.5 mL/well	0.2 mL/well	0.5 mL/well
35-mm dish	10 cm^2	2 mL	1 mL	2 mL
60-mm dish	20 cm^2	4 mL	2 mL	4 mL
100-mm dish	60 cm^2	12 mL	6 mL	12 mL

2. Add 0.5 mM EDTA in DPBS to the dish containing iPSCs. Adjust the volume of EDTA for various dish sizes (refer to Table 3). Swirl the dish to coat the entire cell surface.
3. Incubate the vessel at room temperature for 5–8 minutes or 37°C for 4–5 minutes. When the cells start to separate and round up, and the colonies will appear to have holes in them when viewed under a microscope, they are ready to be removed from the vessel. *Note:* In larger vessels or with certain cell lines, this may take longer than 5 minutes.
4. Aspirate the EDTA solution with a Pasteur pipette.
5. Add pre-warmed complete Essential 8™ Medium to the dish according to Table 3.
6. Remove the cells by gently squirting the colonies from the well using a 5-mL glass pipette. Avoid creating bubbles. Collect cells in a 15-mL conical tube. *Note:* Do not scrape the cells from the dish. There may be obvious patches of cells that were not dislodged and left behind. Do not attempt to recover them through scraping. *Note:* Depending upon the cell line, work with no more than one to three wells at a time, and work quickly to remove cells after adding Essential 8™ Medium to the well(s). The initial effect of the EDTA will be neutralized quickly by the medium. Some lines re-adhere very rapidly after medium addition, and must be removed 1 well at a time. Others are slower to re-attach, and may be removed 3 wells at a time.
7. Centrifuge the cell suspension at 200 × g for 5 minutes to pellet cells.
8. Aspirate and discard the supernatant. Resuspend cell pellet in 500 µL DPBS and transfer resuspended cells to a thin-walled 0.5-mL PCR tube.
9. Centrifuge the cell suspension at 200 x~ g for 5 minutes to pellet cells.
10. Aspirate and discard the supernatant. Resuspend cell pellet in 20 µL of Resuspension Buffer with 2 µL of Lysis Solution added to the Resuspension Buffer.
11. Incubate the cells for 10 minutes in an incubator or thermal cycler that has been preheated to 75°C.
12. Spin the tube briefly to collect any condensation. Use 3 µL of the cell lysate in a 50-µL PCR reaction (see below).

PCR using AccuPrime™ High Fidelity Taq DNA Polymerase

13. Add the following components to a DNase/RNase-free, thin-walled PCR tube as directed in Table 4. Forward and reverse primers are shown in Table 5. For multiple reactions, prepare a master mix of common components to minimize reagent loss and enable accurate pipetting. Note: Assemble PCR reactions in a DNA-free environment. We recommend use of clean dedicated automatic pipettors and aerosol resistant barrier tips.

Table 4 Preparation of reactions for PCR

Component	Volume per reaction
10X PCR Buffer II	5 µL
Forward primer (10 µM stock)	1 µL
Reverse primer (10 µM stock)	1 µL
AccuPrime™ Taq Polymerase (5 units/µL)	1 µL
Cell Lysate	3 µL
Sterile distilled water	39 µL

Table 5 Primers for Standard PCR

Transgene	Primers	Sequence	Expected Size
oriP	pEP4-SF1-oriP pEP4-SR1-oriP	5'-TTC CAC GAG GGT AGT GAA CC-3' 5'-TCG GGG GTG TTA GAG ACA AC-3'	544 bp
EBNA-1	pEP4-SF2-oriP pEP4-SR2-oriP	5'-ATC GTC AAA GCT GCA CAC AG-3' 5'-CCC AGG AGT CCC AGT AGT CA-3'	666 bp

14. *Note:* These primers can detect all three episomal plasmids.
15. Cap the tube, tap gently to mix, and centrifuge briefly to collect the contents.
16. Place the tube in the thermal cycler and use the PCR parameters shown in Table 6:

Table 6 PCR Parameters

Step	Temperature	Time	Cycles
Initial Denaturation	94°C	2 minutes	—
Denaturation	94°C	30 seconds	
Annealing	55°C	30 seconds	35–40
Elongation	72°C	1 minute	
Final Elongation	72°C	7 minutes	—

17. Analyze the PCR products using 2% agarose gel electrophoresis.

Appendix

A. Coating Culture Vessels with Geltrex® LDEV-Free, hESC-Qualified Basement Membrane Matrix

1. Thaw a 5-mL bottle of Geltrex® LDEV-Free hESC-Qualified Reduced Growth Factor Basement Membrane Matrix at 2–8°C overnight.
2. Dilute the thawed Geltrex® solution 1:1 with cold sterile DMEM/F-12 to prepare 1-mL aliquots in tubes chilled on ice. These aliquots can be frozen at –20°C or used immediately.
3. *Note:* Aliquot volumes of 1:1 diluted Geltrex® solution may be adjusted according to your needs.
4. To create working stocks, dilute a Geltrex® aliquot 1:50 with cold DMEM on ice, for a total dilution of 1:100.
   Note: An optimal dilution of the Geltrex® solution may need to be determined for each cell line. Try various dilutions from 1:30 to 1:100.
5. Quickly cover the whole surface of each culture dish with the Geltrex® solution (refer to Table 3).
6. Incubate the dishes in a 37°C, 5% CO2 incubator for 1 hour.
7. Note: Dishes can now be used or stored at 2–8°C for up to a week. Do not allow dishes to dry.
8. Aspirate the diluted Geltrex® solution from the culture dish and discard. You do not need to rinse off the Geltrex® solution from the culture dish after removal. Cells can now be passaged directly onto the Geltrex® matrix-coated culture dish.

Table 7 Volume of Geltrex® hESC-Qualified Matrix Required

Culture Vessel	Surface Area (cm^2)	Volume of Diluted Substrate (mL)
6-well plate	10 cm^2/well	1.5 mL/well
12-well plate	4 cm^2/well	750 µL/well
24-well plate	2 cm^2/well	350 µL/well
35-mm dish	10 cm^2	1.5 mL
60-mm dish	20 cm^2	3.0 mL
100-mm dish	60 cm^2	6.0 mL

B. Cryopreserving iPSCs

1. Pre-warm the required volume of Essential 8™ Medium at room temperature until it is no longer cool to the touch. Do not warm medium in a 37°C water bath.
2. Prepare Essential 8™ Freezing Medium. For every 1 mL of freezing medium needed, aseptically combine the components listed below in a sterile 15-mL tube:

Component	Volume
Complete Essential 8™ Medium	0.9 mL
DMSO	

3. Place the tube with Essential 8™ Freezing Medium on ice until use. Discard any remaining freezing medium after use.
4. Aspirate the spent medium from the dish using a Pasteur pipette, and rinse the cells twice with DPBS without Calcium and Magnesium (refer to Table 3).
5. Add 0.5 mM EDTA solution to the dish. Adjust the volume of EDTA for various dish sizes (refer to Table 4). Swirl the dish to coat the entire cell surface.
6. Incubate the vessel at room temperature for 5–8 minutes or 37°C for 4–5 minutes. When the cells start to separate and round up, and the colonies will appear to have holes in them when viewed under a microscope, they are ready to be removed from the vessel.
7. Aspirate the EDTA solution with a Pasteur pipette.
8. Add 1 mL of ice-cold Essential 8™ Freezing Medium to each well of a 6-well plate.
9. Remove the cells by gently squirting the colonies from the well using a 5-mL glass pipette. Avoid creating bubbles. Collect cells in a 15-mL conical tube on ice.
10. Resuspend cells gently. Aliquot 1 mL of the cell suspension into each cryovial.
11. Quickly place the cryovials in a cryofreezing container (e.g., Mr. Frosty) to freeze the cells at 1°C per minute and transfer them to –80°C overnight.
12. After overnight storage at –80°C, transfer the cells to a liquid nitrogen tank vapor phase for long-term storage.

Reagents suggested:
Invitrogen, Catalog Number: A14666SA
Size 500 ml
List Price: (USD) 195.9

Description

Essential 8™ Medium (published as E8) is a xeno-free and feeder-free medium specially formulated for the growth and expansion of human pluripotent stem cells (PSCs). Originally developed by Guokai Chen et al. (1) in the laboratory of James Thomson (published as "E8") and validated by Cellular Dynamics International, Essential 8™ Medium has been extensively tested and is proven to maintain pluripotency in multiple iPSC lines. In addition, Essential 8™ Medium has been used to scale up production of iPSCs and has been shown to support iPSC growth for >50 passages without any signs of karyotypic abnormalities and maintains the ability of iPSCs to differentiate into all three germ line lineages.

• **Consistent** - Reduced variability compared to existing feeder-free culture media.

• **Robust** - Reliable and robust cultures with a xeno-free, cGMP, 8-component medium.

• **Cost effective** - Economical and scalable PSC culture compared to other feeder-free media.

Note: The "prototype" status of this product means that the product is currently undergoing real time stability studies in compliance with cGMP regulations.

Reduced Variability

Essential 8™ Medium is xeno-free and contains only the eight essential components needed for stem cell culture. Unlike other media that contain over 20 highly variable ingredients, Essential 8™ Medium is produced under cGMP and has an optimized formulation and growth factor levels to help ensure maximum cell health, pluripotency, and growth, with minimal variability.

Reliable and Robust Cultures

Essential 8™ Medium has been show to support pluripotent stem cell growth and provide cultures with superior morphology and growth kinetics compared to other feeder systems.

Cost Effective

Essential 8™ Medium is provided in a convenient two component kit (500 ml basal & 10 ml supplement), and when used with vitronectin (VTN-N), provides a cost effective, defined system for feeder-free culture of human pluripotent stem cells (PSCs).

Essential 8™ Medium is commercialized in partnership with Cellular Dynamics International.

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.
(http://products.invitrogen.com/ivgn/product/A14666SA#).

Literatures

Abraham, S., N. Eroshenko, et al. (2009). "Role of bioinspired polymers in determination of pluripotent stem cell fate." Regen Med **4**(4): 561-78.

Human pluripotent stem cells, including embryonic and induced pluripotent stem cells, hold enormous potential for the treatment of many diseases, owing to their ability to generate cell types useful for therapeutic applications. Currently, many stem cell culture propagation and differentiation systems incorporate animal-derived components for promoting self-renewal and differentiation. However, use of these components is labor intensive, carries the risk of xenogeneic contamination and yields compromised experimental results that are difficult to duplicate. From a biomaterials perspective, the generation of an animal- and cell-free biomimetic microenvironment that provides the appropriate physical and chemical cues for stem cell self-renewal or differentiation into specialized cell types would be ideal. This review

presents the use of natural and synthetic polymers that support propagation and differentiation of stem cells, in an attempt to obtain a clear understanding of the factors responsible for the determination of stem cell fate.

Andrews, P. W., I. Damjanov, et al. (1985). "A pluripotent human stem-cell clone isolated from the TERA-2 teratocarcinoma line lacks antigens SSEA-3 and SSEA-4 in vitro, but expresses these antigens when grown as a xenograft tumor." Differentiation **29**(2): 127-35.

Human embryonal carcinoma (EC) cells generally express the cell-surface, stage-specific embryonic antigens 3 and 4 (SSEA-3 and SSEA-4), the epitopes of which are defined by two monoclonal antibodies that recognize different portions of an extended globoseries oligosaccharide. To examine further the relationship between these epitopes and the human EC phenotype, we investigated the properties of two newly isolated clones from the human teratocarcinoma cell line, TERA-2. One clone expresses SSEA-3 and SSEA-4; the other does not. Nevertheless, these clones otherwise resemble one another, and based upon their morphology, their expression of other cell-surface antigens, and their ability to form xenograft tumors containing a variety of cell types, we conclude that both clones are composed of pluripotent human EC cells. When exposed to retinoic acid in vitro, neither clone differentiates as extensively as other clones that we have previously derived from TERA-2. These observations indicate heterogeneity among stem cells derived from a single human teratocarcinoma, and suggest that SSEA-3 and SSEA-4 are not necessarily integral features of the human EC phenotype. On the other hand, EC cells in xenograft tumors derived from the SSEA-3- and SSEA-4-negative clone re-express these epitopes. Further, this re-expression is stable, since EC cell lines that are SSEA-3- and SSEA-4-positive grow out when the tumors are explanted in vitro. We conclude that the expression of these globoseries epitopes can be modulated by environmental influences.

Astori, G., W. Malangone, et al. (2001). "A novel protocol that allows short-term stem cell expansion of both committed and pluripotent hematopoietic progenitor cells suitable for clinical use." Blood Cells Mol Dis **27**(4): 715-24; discussion 725-7.

To obtain long-term engraftment and hematopoiesis in myeloablated patients, the cell population used for hematopoietic reconstitution should include a sufficient number of early pluripotent hematopoietic stem cells (HSCs), along with committed cells from the various lineages. For this purpose, the small subset of CD34+ cells purified from different sources must be expanded ex vivo. Since cytokines may induce both proliferation and differentiation, expansion would provide a cell population comprising committed as well as uncommitted cells. Optimization of HSC expansion methods could be obtained by a combination of cytokines able to sustain renewal of pluripotent cells yet endowed with poor differentiation potential. We used variations of the combinations of cytokines described by Brugger et al. [W. Brugger, S. Heimfels, R. J. Berenson, R. Mertelsmann, and L. Kanz (1995) N. Engl. J. Med. 333, 283-287] and Piacibello et al. [W. Piacibello, F. Sanavio, L. Garetto, A. Severino, D. Bergandi, J. Ferrario, F. Fagioli, M. Berger, and M. Aglietta (1997) Blood 89, 2644-2653] to expand UCB CD34+ cells and monitored proliferation rate and phenotype after 14 days of culture. Several hematopoietic lineage-associated surface antigens were evaluated. Our data show that flt3L and thrombopoietin in combination with IL-3, while sustaining a high CD34+ proliferation rate, provide a relatively low enrichment in very early uncommitted CD34+/CD38- cells. Conversely, in the absence of IL-3, they are less effective in inducing proliferation yet significantly increase the number of CD34+/CD38- cells. A combination of the above protocols, applied simultaneously to aliquots of the same sample, would allow expansion of both committed and pluripotent HSC. This strategy may represent a significant improvement for clinical applications.

Balasubramanian, S., N. Babai, et al. (2009). "Non cell-autonomous reprogramming of adult ocular progenitors: generation of pluripotent stem cells without exogenous transcription factors." Stem Cells **27**(12): 3053-62.

Direct reprogramming of differentiated cells to induced pluripotent stem (iPS) cells by ectopic expression of defined transcription factors (TFs) represents a significant breakthrough towards the use of stem cells in regenerative medicine (Takahashi and Yamanaka Cell 2006;126:663-676). However, the virus-mediated expression of exogenous transcription factors could be potentially harmful and, therefore, represents a barrier to the clinical use of iPS cells. Several approaches, ranging from plasmid-mediated TF expression to introduction of recombinant TFs (Yamanaka Cell 2009;137:13-17; Zhou, Wu, Joo et al. Cell Stem Cell 2009;4:381-384), have been reported to address the risk associated with viral integration. We describe an alternative strategy of reprogramming somatic progenitors entirely through the recruitment of endogenous genes without the introduction of genetic materials or exogenous factors. To this end, we reprogrammed accessible and renewable

progenitors from the limbal epithelium of adult rat eye by microenvironment-based induction of endogenous iPS cell genes. Non cell-autonomous reprogramming generates cells that are pluripotent and capable of differentiating into functional neurons, cardiomyocytes, and hepatocytes, which may facilitate autologous cell therapy to treat degenerative diseases.

Bendall, S. C., M. H. Stewart, et al. (2007). "IGF and FGF cooperatively establish the regulatory stem cell niche of pluripotent human cells in vitro." Nature **448**(7157): 1015-21.

Distinctive properties of stem cells are not autonomously achieved, and recent evidence points to a level of external control from the microenvironment. Here, we demonstrate that self-renewal and pluripotent properties of human embryonic stem (ES) cells depend on a dynamic interplay between human ES cells and autologously derived human ES cell fibroblast-like cells (hdFs). Human ES cells and hdFs are uniquely defined by insulin-like growth factor (IGF)- and fibroblast growth factor (FGF)-dependence. IGF 1 receptor (IGF1R) expression was exclusive to the human ES cells, whereas FGF receptor 1 (FGFR1) expression was restricted to surrounding hdFs. Blocking the IGF-II/IGF1R pathway reduced survival and clonogenicity of human ES cells, whereas inhibition of the FGF pathway indirectly caused differentiation. IGF-II is expressed by hdFs in response to FGF, and alone was sufficient in maintaining human ES cell cultures. Our study demonstrates a direct role of the IGF-II/IGF1R axis on human ES cell physiology and establishes that hdFs produced by human ES cells themselves define the stem cell niche of pluripotent human stem cells.

Bieberich, E., J. Silva, et al. (2004). "Selective apoptosis of pluripotent mouse and human stem cells by novel ceramide analogues prevents teratoma formation and enriches for neural precursors in ES cell-derived neural transplants." J Cell Biol **167**(4): 723-34.

The formation of stem cell-derived tumors (teratomas) is observed when engrafting undifferentiated embryonic stem (ES) cells, embryoid body-derived cells (EBCs), or mammalian embryos and is a significant obstacle to stem cell therapy. We show that in tumors formed after engraftment of EBCs into mouse brain, expression of the pluripotency marker Oct-4 colocalized with that of prostate apoptosis response-4 (PAR-4), a protein mediating ceramide-induced apoptosis during neural differentiation of ES cells. We tested the ability of the novel ceramide analogue N-oleoyl serinol (S18) to eliminate mouse and human Oct-4(+)/PAR-4(+) cells and to increase the proportion of nestin(+) neuroprogenitors in EBC-derived cell cultures and grafts. S18-treated EBCs persisted in the hippocampal area and showed neuronal lineage differentiation as indicated by the expression of beta-tubulin III. However, untreated cells formed numerous teratomas that contained derivatives of endoderm, mesoderm, and ectoderm. Our results show for the first time that ceramide-induced apoptosis eliminates residual, pluripotent EBCs, prevents teratoma formation, and enriches the EBCs for cells that undergo neural differentiation after transplantation.

Bodine, D. M., N. E. Seidel, et al. (1994). "Efficient retrovirus transduction of mouse pluripotent hematopoietic stem cells mobilized into the peripheral blood by treatment with granulocyte colony-stimulating factor and stem cell factor." Blood **84**(5): 1482-91.

Cytokine-mobilized peripheral blood cells have been shown to participate in hematopoietic recovery after bone marrow (BM) transplantation, and are proposed to be useful targets for retrovirus-mediated gene transfer protocols. We treated mice with granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) to mobilize hematopoietic progenitor cells into the peripheral blood. These cells were analyzed for the number and frequency of pluripotent hematopoietic stem cells (PHSC). We found that splenectomized animals treated for 5 days with G-CSF and SCF showed a threefold increase in the absolute number of PHSC over normal mice. The number of peripheral-blood PHSC increased 250-fold from 29 per untreated mouse to 7,200 in peripheral-blood PHSC in splenectomized animals treated for 5 days with G-CSF and SCF. Peripheral blood PHSC mobilized by treatment with G-CSF and SCF were analyzed for their ability to be transduced by retroviral vectors. Peripheral-blood PHSC from splenectomized animals G-CSF and SCF were transduced with a recombinant retrovirus containing the human MDR-1 gene. The frequency of gene transfer into peripheral blood PHSC from animals treated for 5 and 7 days was two-fold and threefold higher than gene transfer into PHSC from the BM of 5-fluorouracil-treated mice ($P < .01$). We conclude that peripheral blood stem cells mobilized by treatment with G-CSF and SCF are excellent targets for retrovirus-mediated gene transfer.

Boiani, M., L. Gentile, et al. (2005). "Variable reprogramming of the pluripotent stem cell marker Oct4 in mouse clones: distinct developmental potentials in different culture environments." Stem Cells **23**(8): 1089-104.

A prevailing view of cloning by somatic-cell nuclear transfer is that reprogramming of gene expression occurs during the first few hours after

injection of the nucleus into an oocyte, that the process is stochastic, and that the type of reprogramming needed for cloning success is foreign and unlikely to be readily achieved in the ooplasm. Here, we present evidence that the release of reprogramming capacity is contingent on the culture environment of the clone while the contribution of aneuploidy to altered gene expression is marginal. In particular, the rate of blastocyst formation in clones and the regional distribution of mRNA for the pluripotent stem cell marker Oct4 in clonal blastocysts was highly dependent on the culture environment after cumulus cell nuclear transfer, unlike that in genetically equivalent zygotes. Epigenetic modifications of genetically identical somatic nuclei continue after the first cell division of the clones and are amenable to a degree of experimental control, and their development to the blastocyst stage and appropriate expression of Oct4 predict further outcome, such as derivation of embryonic stem (ES) cells, but not fetal development. This observation indicates that development to the blastocyst stage is not equivalent to full reprogramming and lends support to the novel concept that ES cells are not the equivalent of the inner cell mass, hence the discrepancy between ES cell derivability and fetal development of clones.

Chen, S., A. Choo, et al. (2006). "TGF-beta2 allows pluripotent human embryonic stem cell proliferation on E6/E7 immortalized mouse embryonic fibroblasts." J Biotechnol **122**(3): 341-61.

In this study we report observations that mouse embryonic fibroblasts (MEF) capable of supporting expansion of pluripotent, human embryonic stem cells (hESC) fail to support after immortalization using E6/E7 oncogenes in serum conditions; however this can be reversed following addition of exogenous TGF-beta2. Microarray analysis of immortalized and non-immortalized MEF revealed differential gene expression of several TGF-beta related genes. By supplementing TGF-beta2 into E6/E7 immortalized MEF cultures, this enabled proliferation of undifferentiated, pluripotent hESC as demonstrated by marker expression (Oct-4, SSEA-4, alkaline phosphatase) and teratoma formation representing three germ layers following hESC injection into immuno-deficient mice. Subsequent investigation using quantitative real-time PCR highlighted differential gene expression of several extracellular matrix related transcripts in primary and immortal (+/-TGF-beta2) feeder cells including the induction of osteopontin following addition of TGF-beta2. Our results demonstrate that TGF-beta2 and its related genes in MEF play a role in the support of pluripotent hESC expansion.

Choi, K. D., M. A. Vodyanik, et al. (2009). "Generation of mature human myelomonocytic cells through expansion and differentiation of pluripotent stem cell-derived lin-CD34+CD43+CD45+ progenitors." J Clin Invest **119**(9): 2818-29.

Basic research into human mature myelomonocytic cell function, myeloid lineage diversification and leukemic transformation, and assessment of myelotoxicity in preclinical drug development requires a constant supply of donor blood or bone marrow samples and laborious purification of mature myeloid cells or progenitors, which are present in very small quantities. To overcome these limitations, we have developed a protocol for efficient generation of neutrophils, eosinophils, macrophages, osteoclasts, DCs, and Langerhans cells from human embryonic stem cells (hESCs). As a first step, we generated lin-CD34+CD43+CD45+ hematopoietic cells highly enriched in myeloid progenitors through coculture of hESCs with OP9 feeder cells. After expansion in the presence of GM-CSF, these cells were directly differentiated with specific cytokine combinations toward mature cells of particular types. Morphologic, phenotypic, molecular, and functional analyses revealed that hESC-derived myelomonocytic cells were comparable to their corresponding somatic counterparts. In addition, we demonstrated that a similar protocol could be used to generate myelomonocytic cells from induced pluripotent stem cells (iPSCs). This technology offers an opportunity to generate large numbers of patient-specific myelomonocytic cells for in vitro studies of human disease mechanisms as well as for drug screening.

Conway, A. E., A. Lindgren, et al. (2009). "A self-renewal program controls the expansion of genetically unstable cancer stem cells in pluripotent stem cell-derived tumors." Stem Cells **27**(1): 18-28.

Human germ cell tumors are often metastatic, presumably due to distal site tumor growth by cancer stem cells. To determine whether cancer stem cells can be identified in a transplantation model of testicular germ cell tumor, we transplanted murine embryonic germ cells (EGCs) into the testis of adult severe combined immunodeficient mice. Transplantation resulted in a locally invasive solid tumor, with a cellular component that generated secondary tumors upon serial transplantation. The secondary tumors were invariably metastatic, a feature not observed in the primary tumors derived from EGCs. To characterize the differences between EGCs and the tumor-derived stem cells, we performed karyotype and microarray analysis. Our results show that generation of cancer stem cells is associated with

the acquisition of nonclonal genomic rearrangements not found in the originating population. Furthermore, pretreatment of EGCs with a potent inhibitor of self-renewal, retinoic acid, prevented tumor formation and the emergence of these genetically unstable cancer stem cells. Microarray analysis revealed that EGCs and first- and second-generation cancer stem cells were highly similar; however, approximately 1,000 differentially expressed transcripts could be identified corresponding to alterations in oncogenes and genes associated with motility and development. Combined, the data suggest that the activation of oncogenic pathways in a cellular background of genetic instability, coupled with an inherent ability to self-renew, is involved in the acquisition of metastatic behavior in the cancer stem cell population of tumors derived from pluripotent cells.

Cram, D. S., B. Song, et al. (2007). "Genotyping of Rhesus SCNT pluripotent stem cell lines." Nature **450**(7169): E12-4.

Somatic cell nuclear transfer (SCNT) into enucleated oocytes has emerged as a technique that can be used to derive mouse embryonic stem cell lines with defined genotypes. In this issue Byrne et al. report the derivation of two SCNT Rhesus macaca male stem cell lines designated CRES-1 and CRES-2. Molecular studies detailed in their paper provides supporting evidence that the chromosome complement of CRES-1 and CRES-2 was genetically identical to the male cell donor nucleus and that the mitochondrial DNA originated from different recipient oocytes. In this validation paper, we independently confirm that both stem cell lines were indeed derived by SCNT.

Daley, G. Q. (2007). "Towards the generation of patient-specific pluripotent stem cells for combined gene and cell therapy of hematologic disorders." Hematology Am Soc Hematol Educ Program: 17-22.

Hematopoietic stem cell transplantation (HSCT) has proven successful for the treatment of a host of genetic and malignant diseases of the blood, but immune barriers to allogeneic tissue transplantation have hindered wider application. Likewise, gene therapy now appears effective in the treatment of various forms of immune deficiency, and yet insertional mutagenesis from viral gene transfer has raised safety concerns. One strategy for addressing the limitations of both gene therapy and allogeneic transplantation entails the creation of pluripotent stem cells from a patient's own somatic cells, thereby enabling precise in situ gene repair via homologous recombination in cultured cells, followed by autologous tissue transplantation. In murine model systems, the methods of somatic cell nuclear transfer, parthenogenesis, and direct somatic cell reprogramming with defined genetic factors have been used to generate pluripotent stem cells, and initial efforts at therapeutic gene repair and tissue transplantation suggest that the technology is feasible. Generating patient-specific autologous pluripotent stem cells provides an opportunity to combine gene therapy with autologous cell therapy to treat a host of human conditions. However, a number of technical hurdles must be overcome before therapies based on pluripotent human stem cells will appear in the clinic.

David, R., J. Stieber, et al. (2009). "Forward programming of pluripotent stem cells towards distinct cardiovascular cell types." Cardiovasc Res **84**(2): 263-72.

AIMS: The proliferative potential of pluripotent stem cell-derived cardiomyocytes is limited, and reasonable yields for novel therapeutic options have yet to be achieved. In addition, various clinical applications will require the generation of specific cardiac cell types. Whereas early cardiovascular precursors appear to be important for novel approaches such as reseeding decellularized hearts, direct cell transplantation may require ventricular cells. Our recent work demonstrated that MesP1 represents a master regulator sufficient to induce cardiovasculogenesis in pluripotent cells. This led to our hypothesis that 'forward programming' towards specific subtypes may be feasible via overexpression of distinct early cardiovascular transcription factors. METHODS AND RESULTS: Here we demonstrate that forced expression of Nkx2.5 similar to MesP1 is sufficient to enhance cardiogenesis in murine embryonic stem cells (mES). In comparison to control transfected mES cells, a five-fold increased appearance of beating foci was observed as well as upregulated mRNA and protein expression levels. In contrast to MesP1, no increase of the endothelial lineage within the cardiovasculogenic mesoderm was observed. Likewise, Flk-1, the earliest known cardiovascular surface marker, was not induced via Nkx2.5 as opposed to MesP1. Detailed patch clamping analyses showed electrophysiological characteristics corresponding to all subtypes of cardiac ES cell differentiation in Nkx2.5 as well as MesP1 programmed embryoid bodies, but fractions of cardiomyocytes had distinct characteristics: MesP1 forced the appearance of early/intermediate type cardiomyocytes in comparison to control transfected ES cells whereas Nkx2.5 led to preferentially differentiated ventricular cells. CONCLUSION: Our findings show proof of principle for cardiovascular subtype-specific programming of pluripotent stem cells and confirm the molecular hierarchy for cardiovascular specification initiated via MesP1 with

differentiation factors such as Nkx2.5 further downstream.

Delaney, C. and I. D. Bernstein (2004). "Establishment of a pluripotent preleukaemic stem cell line by expression of the AML1-ETO fusion protein in Notch1-immortalized HSCN1cl10 cells." Br J Haematol **125**(3): 353-7.

The AML1-ETO fusion has been associated with up to 40% of acute myeloid leukaemia French-American-British classified M2 cases. This chimaeric protein interferes with normal AML1 function and disrupts critical transcriptional regulation of haematopoiesis. Current evidence suggests that AML1-ETO alone is insufficient to induce leukaemia, but rather is a co-operating event in leukaemogenesis. We developed a pluripotent murine haematopoietic stem cell line expressing the AML1-ETO fusion protein that displays in vitro and in vivo properties consistent with a preleukaemic state, including inhibition of terminal granulocytic differentiation in vitro and the development of non-lymphoid leukaemias in vivo. This cell line represents a potential platform for the introduction and in vitro rapid screening of candidate genes thought to co-operate with AML1-ETO in developing frank leukaemia.

Desponts, C., J. M. Ninos, et al. (2006). "s-SHIP associates with receptor complexes essential for pluripotent stem cell growth and survival." Stem Cells Dev **15**(5): 641-6.

Embryonic stem (ES) cells are pluripotent cells that have the ability to either self-renew or differentiate into any cell type found in the mammalian body. The signaling pathways required for self-renewal of these cells are yet to be defined. Previously we identified a stem cell-specific isoform of the protein SH2 domain-containing 5'-inositol phosphatase (SHIP) that we call s-SHIP, which is expressed in both pluripotent ES cells and adult tissue-specific multipotent cells, such as hematopoietic stem cells (HSCs). s-SHIP lacks an SH2 domain but contains a 5'-inositol phosphatase domain and several protein-protein interaction domains that potentially enable its participation in many different signaling pathways. Here we show that s-SHIP associates with gp130, which forms a heterodimeric complex with the leukemia inhibitory factor receptor (LIFR). Signaling through LIFR and other receptors that heterodimerize with gp130 is critical for growth and survival of ES cells and HSCs. Our findings provide biochemical evidence that s-SHIP participates in signaling pathways important for the maintenance of pluripotent stem cell populations.

Dumenil, D., F. Sainteny, et al. (1982). "Modifications of pluripotent stem cell differentiation after ARA-C treatment: clonal analyses of CFU-S progeny." Leuk Res **6**(6): 753-60.

The nature of the mechanisms controlling CFU-S differentiation is a crucial problem in haematology and, thus far, little is known concerning these phenomena. Work done in our laboratory has shown that the distribution of the histologic cell types represented in spleen colonies (CFU-S) differ depending on whether normal bone marrow or marrow from Ara-C treated mice is injected into the irradiated recipients. As measured by the mean of the absolute number of colonies per spleen, bone marrow from Ara-C treated mice gives more erythroid colonies and fewer granulocytic colonies than do cells from normal bone marrow. We have demonstrated that these modifications are under the control of humoral factors. Two significant questions arise from these observations. First, are the colonies after Ara-C treatment derived from a single multi-potential cell rather than from already committed progenitors and, second, is this shift in granulocytic-erythroid representation a reflection of modifications at the CFU-S level introduced by our Ara-C system? To answer these questions, we analysed the progeny of each individual spleen nodule either by reinjecting each colony unit into a secondary recipient or by cloning these cells in methyl cellulose with appropriate stimulating factors. We thus determined the number of retransplantable stem cells, as well as the number of committed precursors present in each spleen nodule. Our results demonstrate that most spleen colonies are transplantable and give rise to secondary colonies. These secondary colonies are of all haematological types, therefore proving that the nodules contain CFU-S and that these CFU-S are pluripotent. All spleen colonies contain GM-CFC, even in the nodules that were histologically erythroid. We thus conclude that modifications in the E/G ratio of spleen colonies after injection of bone marrow from Ara-C treated mice are a reflection of changes in CFU-S differentiation pathways.

Edwards, B. E., J. D. Gearhart, et al. (2000). "The human pluripotent stem cell: impact on medicine and society." Fertil Steril **74**(1): 1-7.

OBJECTIVE: To discuss the current state of the science surrounding human pluripotent stem cells and to show that the derivation of such cells from donated preimplantation human embryos should be eligible for federal funding provided that certain protections are met. DESIGN: A literature search focusing on the scientific aspects of pluripotent stem-cell research and analyses of current and past legislation and federal panel recommendations.

CONCLUSION(S): The current federal laws regulating the permission necessary to obtain fetal tissue from elective pregnancy terminations are intended to insulate the decision to terminate a pregnancy from the potential positive influence of fetal tissue transplantation. A similar situation can be created for the derivation of cells from excess preimplantation human embryos produced by IVF programs. If, as in fetal tissue research, assurances can be made that the research will have no influence on the decision to dispose of the embryo, the derivation of pluripotent stem cells from embryo should proceed with federal funding.

Eistetter, H. R. (1988). "A mouse pluripotent embryonal stem cell line stage-specifically regulates expression of homeo-box containing DNA sequences during differentiation in vitro." Eur J Cell Biol **45**(2): 315-21.

Mouse embryonal stem (ES) cells have been shown to provide a new model system suitable for the analysis of different aspects of murine development. This report gives evidence that ES cell lines are also most useful for the study of developmentally regulated gene expression in vitro. Homeo-box containing genes which are suggested to play a key role in the regulation of differentiation steps occurring during embryogenesis are stage-specifically transcribed in differentiating murine ES cells: (i) A mouse embryonal stem cell line (ES-12957) was isolated and characterized with respect to its differentiation potential. When injected subcutaneously into syngeneic mice, ES-12957 cells formed fully differentiated teratomas representing derivatives of all three germ layers. When allowed to grow in suspension cultures in vitro, the cells followed a reproducible developmental pathway forming complex organized 'embryoid bodies' which resembled mouse early postimplantation embryos. (ii) A mouse DNA sequence with homeo-box homology (MH-121) was isolated and structurally analyzed. Transcription of a 1.7 kb RNA species from this DNA sequence was demonstrated in ES-12957 cells which were differentiated in vitro. A second, previously described homeo-box gene (Mo-10) was also shown to be expressed in ES-12957 cells in a stage-specific manner. A 4-kb transcript could be identified exclusively in RNA of cells which were allowed to differentiate for 9 days. These findings support the suggestion that the homeo-box genes of mammals, like those of Drosophila, may have important functions during embryonic development.

Esteban, M. A., J. Xu, et al. (2009). "Generation of induced pluripotent stem cell lines from Tibetan miniature pig." J Biol Chem **284**(26): 17634-40.

Induced pluripotent stem cell (iPS) technology appears to be a general strategy to generate pluripotent stem cells from any given mammalian species. So far, iPS cells have been reported for mouse, human, rat, and monkey. These four species have also established embryonic stem cell (ESC) lines that serve as the gold standard for pluripotency comparisons. Attempts have been made to generate porcine ESC by various means without success. Here we report the successful generation of pluripotent stem cells from fibroblasts isolated from the Tibetan miniature pig using a modified iPS protocol. The resulting iPS cell lines more closely resemble human ESC than cells from other species, have normal karyotype, stain positive for alkaline phosphatase, express high levels of ESC-like markers (Nanog, Rex1, Lin28, and SSEA4), and can differentiate into teratomas composed of the three germ layers. Because porcine physiology closely resembles human, the iPS cells reported here provide an attractive model to study certain human diseases or assess therapeutic applications of iPS in a large animal model.

Fabian, I., D. Douer, et al. (1985). "Human spleen cell generation of factors stimulating human pluripotent stem cell, erythroid, and myeloid progenitor cell growth." Blood **65**(4): 990-6.

Mitogen-stimulated murine spleen cells produce humoral substances capable of supporting murine hematopoiesis and pluripotent stem cell proliferation in vitro. Thus, we evaluated conditioned media generated by human spleen cells (SCM) in the presence or absence of mitogens for factors stimulatory for human pluripotent (CFU-GEMM), erythroid (BFU-E), and myeloid (CFU-GM) precursors. Two and one half percent to 10% SCM stimulated proliferation of all three types of precursor cells from nonadherent buoyant human marrow target cells. Mitogen-stimulated SCM augmented CFU-GM (175% to 225%), whereas CFU-GEMM and BFU-E growth was essentially unchanged. Cell separation procedures used to determine which cells provided these microenvironmental stimuli indicated that nonadherent mononuclear spleen cells provided the bulk of the CSF-GM, whereas adherent cells (95% nonspecific esterase + monocyte-macrophages) and nonadherent cells provided similar proportions of CSF-mix and erythroid burst-promoting activity (BPA). The nonadherent cells generating high levels of CSF-mix, BPA, and CSF-GM were predominantly Leu-1-negative, ie, non-T, cells. In the presence or absence of mitogens, SCM was a more potent source (1.3- to 3.8-fold) than peripheral leukocyte CM of the growth factors for the three progenitor cell types. Specific in situ cytochemical stains for analyzing morphology of myeloid colonies demonstrated that

SCM stimulated the proliferation of the same types and proportions of colonies as human placental CM, suggesting that these CMs may contain similar CSF-GMs. These data show the contribution of spleen cell subsets to the generation of hematopoietic growth factors and the responsiveness of these cells to various mitogenic stimuli.

Fauser, A. A., K. G. Bross, et al. (1982). "Suppressor T-cell clones derived from pluripotent stem cells (CFU-GEMM) of a patient with Hodgkin's lymphoma." Blut **45**(2): 97-102.

Pluripotent stem cells (CFU-GEMM) give rise to multilineage hemopoietic colonies in culture. The cellular composition revealed that mixed colonies contain cells of different myeloid lineages and mononuclear cells with T-cell surface antigens. T-lymphocytes of primary colonies, replated secondary and tertiary colonies from a patient with Hodgkin's Lymphoma were identified by their reaction with the monoclonal antibody OKT 8. Evidence for a common progenitor of myeloid and lymphoid cells is provided by analysis of individual secondary and tertiary colonies using OKT 3, OKT 4, OKT 8, VIM-D 5, and Ig M + D antibodies for each individual colony. Primary mixed, replated secondary and tertiary colonies revealed OKT 8 positive cells. No reaction with OKT 3, OKT 4, VIM-D 5, or Ig M + D was observed.

Freund, C. and C. L. Mummery (2009). "Prospects for pluripotent stem cell-derived cardiomyocytes in cardiac cell therapy and as disease models." J Cell Biochem **107**(4): 592-9.

The derivation of embryonic stem cells (hESC) from human embryos a decade ago started a new era in perspectives for cell therapy as well as understanding human development and disease. More recently, reprogramming of somatic cells to an embryonic stem cell-like state (induced pluripotent stem cells, iPS) presented a new milestone in this area, making it possible to derive all cells types from any patients bearing specific genetic mutations. With the development of efficient differentiation protocols we are now able to use the derivatives of pluripotent stem cells to study mechanisms of disease and as human models for drug and toxicology testing. In addition derivatives of pluripotent stem cells are now close to be used in clinical practice although for the heart, specific additional challenges have been identified that preclude short-term application in cell therapy. Here we review techniques presently used to induce differentiation of pluripotent stem cells into cardiomyocytes and the potential these cells have as disease models and for therapy.

Goldschneider, I., D. Metcalf, et al. (1980). "Analysis of rat hemopoietic cells on the fluorescence-activated cell sorter. I. Isolation of pluripotent hemopoietic stem cells and granulocyte-macrophage progenitor cells." J Exp Med **152**(2): 419-37.

A scheme is presented whereby pluripotent hemopoietic stem cells (PHSC) from rat bone marrow can be enriched 320-fold with the aid of the fluorescence- activated cell sorter. This scheme is based on the observations that PHSC are strongly positive for Thy-1 antigen (upper 10th percentile); have light- scattering properties (size distribution) between those of bone marrow lymphocytes and myeloid progenitor cells; and are relatively resistant to cortisone. It is estimated that PHSC may constitute 80 percent of the cells isolated according to these parameters. Candidate PHSC are described at the light and electron microscopic levels. At least two populations of accessory cells appear to influence the number and/or the nature of the hemopoietic colonies that form in the in vivo spleen colony-forming unit assay. Putative amplifier cells are strongly Thy-1(+) and cortisone sensitive; putative suppressor cells are weakly Thy-1(+) and cortisone resistant. Three subsets of granulocyte (G) -macrophage (M) progenitor cells (in vitro colony-forming cells [CFC]) are identified on the basis of relative fluorescence intensity for Thy-1 antigen: G-CFC are strongly Thy-l(+); M-CFC are weakly Thy-l(+); and cells that produce mixed G and M CFC have intermediate levels of Thy-1. GM-cluster-forming cells and mature G and M are Thy-1(-). The results suggest that G-CFC are bipotential cells that give rise to G and M-CFC; and that the latter produce mature M through a cluster-forming cell intermediate. Thy-1 antigen is also demonstrated on members of the eosinophil, megakaryocyte, erythrocyte, and lymphocyte cell series in rat bone marrow. In each instance, the relative concentration of Thy-1 antigen is inversely related to the state of cellular differentiation.

Gordeeva, O., R. Zinovieva, et al. (2005). "Differentiation of embryonic stem cells after transplantation into peritoneal cavity of irradiated mice and expression of specific germ cell genes in pluripotent cells." Transplant Proc **37**(1): 295-8.

Permanent embryonic stem cell lines (ES cells) are considered as one of the most promising cellular sources for regenerative medicine. ES cells have a high proliferative potency and ability to differentiate into all kinds of somatic and germ cells. However, transplantation of undifferentiated ES cells into adult recipient tissue results in the formation of teratomas. To understand the mechanisms underlying self-renewal and determination of pluripotent cells, we investigated differentiation potencies of

undifferentiated ES cells and differentiating embryoid bodies (EB). ES cells and EBs growing on acetate-cellulose membranes were transplanted into the peritoneal cavity of irradiated mice. Behavior and differentiation of transplanted cells were studied within 1, 2, 3, and 6 weeks after transplantation. No differences in the cell composition were found in the teratomas formed by ES cells and differentiating EBs. The pattern of expression of the genes specific for pluripotent and germ cells was studied in all types of experimental teratomas. The expression of oct4, stella, fragilis was detected in the teratomas, but nanog was not expressed. We conclude that pluripotent cells are retained in the experimental teratomas formed after transplantation of ES cells and EBs but the pattern of expression of the studied genes underwent changes.

Guigon, M., J. Y. Mary, et al. (1982). "Protection of mice against lethal doses of 1 beta-D-arabinofuranosylcytosine by pluripotent stem cell inhibitors." Cancer Res **42**(2): 638-41.

The aim of this work was to study whether an inhibitor of pluripotent stem cell (CFU-S) recruitment, which we have shown previously to be able to increase the number of CFU-S after a fractionated treatment with 1-beta-D-arabinofuranosylcytosine, could increase the survival of mice given injections of lethal doses of the same drug. Two protocols of 1-beta-D-arabinofuranosylcytosine treatment were used in two different mouse strains, which both killed the mice within a week. An inhibitor of CFU-S was prepared by dialysis from fetal calf marrow, and a first step of purification was made by chromatography on Sephadex G-10. When given injections 2 hr before the drug, the number of surviving mice was increased significantly with the dialysate; fractions separated by chromatography appeared to be more effective to increase the animal survival. These preliminary results indicate that a factor of low molecular weight (below M.W. 3500) extracted from fetal calf marrow is able to protect animals during 1-beta-D-arabinofuranosylcytosine treatment. The inhibitor seems to be specific for CFU-S, without any inhibiting effect on tumor cell kinetics in vitro. If the absence of species specificity found for higher to lower species is confirmed for the lower to the higher species, then this inhibitor could be an effective tool during cancer chemotherapy.

Gunaratne, P. H. (2009). "Embryonic stem cell microRNAs: defining factors in induced pluripotent (iPS) and cancer (CSC) stem cells?" Curr Stem Cell Res Ther **4**(3): 168-77.

The discovery of microRNAs (miRNAs - small non-coding RNAs of approximately 22 nt) heralded a new and exciting era in biology. During this period miRNAs have gone from ignominy due to their origin mainly in 'junk DNA' to notoriety where they can be at once characterized as being all powerful (a single miRNA can target and potentially silence several hundred genes) and yet marginal (a given gene can be targeted by several miRNAs such that a given miRNA typically exerts a modest repression) [1-4]. The emerging paradox is exemplified by miRNAs that are prominently expressed in embryonic stem (ES) cells. The collective importance of miRNAs is firmly established by the fact that Dicer-/- mouse embryos die on day 7.5 due to defects in differentiation [5]. However, oppositely correlated expression that is expected of conventional repressors is increasingly being defied in multiple systems in relation to miRNA-mRNA target pairs. This is most evident in ES cells where miR-290-295 and 302 clusters the most abundant ES cell miRNAs are found to be driven by pluripotency genes Oct4, Nanog and Sox2 and also target these genes in 'incoherent feed-forward loops' [7]. Here the miRNAs are co-expressed and positively correlated with these targets that they repress suggesting that one of their primary roles is to fine tune gene expression rather than act as ON/OFF switches. On the other hand, let-7 family members that are notably low in ES cells and rapidly induced upon differentiation exhibit more conventional anti-correlated expression patterns with their targets [7-8]. In an intricately designed auto-regulatory loop, LIN28, a key 'keeper' of the pluripotent state binds and represses the processing of let-7 (a key 'keeper' of the differentiated state) [9-11]. One of the let-7 family members, let-7g targets and represses LIN28 through four 3'-UTR binding sites [12]. We propose that LIN28/let-7 pair has the potential to act as a 'toggle switch' that balances the decision to maintain pluripotency vs. differentiation. We also propose that the c-Myc/E2F driven miR17-92 cluster that together controls the G1 to S transition is fundamental for ES self-renewal and cell proliferation [13-18]. In that context it is no surprise that LIN28 and c-Myc (and therefore let-7 and miR-17-92 by association) and more recently Oct4/Sox2 regulated miR-302 has been shown to be among a handful of factors shown to be necessary and sufficient to convert differentiated cells to induced pluripotent stem (iPS) cells [19-29]. It is also no surprise that activation of miR-17-92 (OncomiRs) and down-regulation of let-7 (tumor suppressors) is a recurring theme in relation to cancers from multiple systems [30-48]. We speculate that the LIN28/let-7; c-MYC-E2F/miR-17-92 and Oct4/Sox2/miR-302-cyclin D1 networks are fundamental to properties of pluripotency and self-renewal associated with embryonic stem cells. We also speculate that ES cell miRNA-mRNA associations may also regulate tissue homeostasis and

regeneration in the fully developed adult. Consequently, the appropriate regulation of LIN28/let-7; c-MYC-E2F/miR-17-92 and Oct4/Sox2/miR-302-cyclin D1 gene networks will be critical for the success of regenerative strategies that involve iPS cells. Any perturbation in key ES cell miRNA-mRNA networks during any of the above processes maybe a hallmark of (CSCs).

Hamaguchi, H., Y. Nakamura, et al. (1993). "Philadelphia-chromosome-positive, monosomy 7 biphenotypic acute mixed lineage leukemia in adults: a pluripotent stem cell disorder." Leukemia **7**(11): 1752-8.

Two adult patients with acute mixed lineage leukemia (AMLL) having combined Philadelphia chromosome (Ph1) positivity and monosomy 7 are presented. The phenotypes of leukemic blasts from both cases were almost same (early B-lymphoid lineage and myeloid lineage); CD10+, CD13+, CD19+. HLA-DR+, and dual-color analysis showed simultaneous expression of CD10 (CD19) and CD13 antigens in individual blasts (biphenotypic) in both cases. On molecular analysis, the leukemic blasts showed rearrangement in the first intron of the BCR gene with breakpoint just outside of 3' end of m-BCR-2 (bcr 3) in case 1, and in the M-BCR in case 2. Immunoglobulin heavy chain gene (IgH) rearrangement was noted in both cases, but rearrangement of the T-cell receptor beta-chain gene (TCR beta) was detected only in case 1. Clinically, both cases achieved complete remission by the combination chemotherapy consisting of L-asparaginase, doxorubicin, vincristine, and prednisolone (L-AdVP). In remission, all these molecular abnormalities disappeared in both patients. These results suggest that the Ph1-positive and monosomy 7 AMLL in adults is de novo acute leukemia with both early B-lymphoid and myeloid phenotypes and may arise from malignant transformation of pluripotent stem cell, and expresses a heterogenous rearrangement pattern of the BCR gene.

Harb, N., T. K. Archer, et al. (2008). "The Rho-Rock-Myosin signaling axis determines cell-cell integrity of self-renewing pluripotent stem cells." PLoS One **3**(8): e3001.

BACKGROUND: Embryonic stem (ES) cells self-renew as coherent colonies in which cells maintain tight cell-cell contact. Although intercellular communications are essential to establish the basis of cell-specific identity, molecular mechanisms underlying intrinsic cell-cell interactions in ES cells at the signaling level remain underexplored. METHODOLOGY/PRINCIPAL FINDINGS: Here we show that endogenous Rho signaling is required for the maintenance of cell-cell contacts in ES cells. siRNA-mediated loss of function experiments demonstrated that Rock, a major effector kinase downstream of Rho, played a key role in the formation of cell-cell junctional assemblies through regulation of myosin II by controlling a myosin light chain phosphatase. Chemical engineering of this signaling axis by a Rock-specific inhibitor revealed that cell-cell adhesion was reversibly controllable and dispensable for self-renewal of mouse ES cells as confirmed by chimera assay. Furthermore, a novel culture system combining a single synthetic matrix, defined medium, and the Rock inhibitor fully warranted human ES cell self-renewal independent of animal-derived matrices, tight cell contacts, or fibroblastic niche-forming cells as determined by teratoma formation assay. CONCLUSIONS/SIGNIFICANCE: These findings demonstrate an essential role of the Rho-Rock-Myosin signaling axis for the regulation of basic cell-cell communications in both mouse and human ES cells, and would contribute to advance in medically compatible xeno-free environments for human pluripotent stem cells.

Hellmen, E., M. Moller, et al. (2000). "Expression of different phenotypes in cell lines from canine mammary spindle-cell tumours and osteosarcomas indicating a pluripotent mammary stem cell origin." Breast Cancer Res Treat **61**(3): 197-210.

Mammary spindle-cell tumours and sarcomas seem to be restricted to dogs and humans. Two cell lines from spontaneous primary canine mammary spindle-cell tumours (CMT-U304 and CMT-U309) and two cell lines from spontaneous primary canine mammary osteosarcomas (CMT-U334 and CMT-U335) were established to study the mesenchymal phenotypes of mammary tumours in the female dog. The cells from the spindle-cell tumours expressed cytokeratin, vimentin and smooth muscle actin filaments. When these cells were inoculated subcutaneously into female and male nude mice they formed different types of mesenchymal tumours such as spindle-cell tumours, fibroma and rhabdomyoid tumours (n = 6/8). The cells from the osteosarcomas expressed vimentin filaments and also formed different types of mesenchymal tumours such as chondroid, rhabdomyoid, smooth muscle-like and spindle-cell tumours (n = 6/10). The cell lines CMT-U304, CMT-U309 and CMT-U335 had receptors for progesterone but none of the four cell lines had receptors for estrogen. All four cell lines and their corresponding primary tumours showed identical allelic patterns in microsatellite analysis. By in situ hybridization with genomic DNA we could verify that all formed tumours but one were of canine origin. Our

results support the hypothesis that canine mammary tumours are derived from pluripotent stem cells.

Hogge, D. E., K. M. Shannon, et al. (1987). "Juvenile monosomy 7 syndrome: evidence that the disease originates in a pluripotent hemopoietic stem cell." Leuk Res **11**(8): 705-9.

The present study was undertaken to investigate the hemopoietic cell from which malignant change evolves in juvenile dyshemopoiesis with monosomy 7. Two male patients, aged 18 and 5 months, were studied using progenitor assays combined with cytogenetics. Both had hepatosplenomegaly, cytopenias and a cellular marrow. The karyotype in direct marrow was 45,XY-7/47,XY,+8/46,XY in patient 1 and 45,XY,-7/46,XY in patient 2. Patient 1 received chemotherapy but developed acute nonlymphocytic leukemia after 17 months and died 20 months after diagnosis. During this time marrow metaphases with 45,XY,-7 increased to 100% (25/25). Patient 2 received an allogeneic marrow transplant 4 months after diagnosis which did not engraft. In both patients progenitors of both small (CFU-E) and large (BFU-E) erythroid colonies were present at normal frequencies. However, the colonies produced were small and poorly hemoglobinized with some erythropoietin-independent maturation. Progenitors of large granulocyte/macrophage colonies (CFU-GM) were present at an elevated frequency in the marrow of patient 1 and in the blood all progenitor classes were markedly increased. Cytogenetic analysis of colonies from this patient showed BFU-E to be 45,XY,-7 or 47,XY,+8 and CFU-GM to be 45,XY,-7 or 47,XY,+8 or 46,XY. In patient 2, most BFU-E were 45,XY,-7, although a few were 46,XY. These data indicate that malignant change in this disease involves hemopoietic stem cells capable of erythroid and in at least some cases, myeloid differentiation.

Hong, H., K. Takahashi, et al. (2009). "Suppression of induced pluripotent stem cell generation by the p53-p21 pathway." Nature **460**(7259): 1132-5.

Induced pluripotent stem (iPS) cells can be generated from somatic cells by the introduction of Oct3/4 (also known as Pou5f1), Sox2, Klf4 and c-Myc, in mouse and in human. The efficiency of this process, however, is low. Pluripotency can be induced without c-Myc, but with even lower efficiency. A p53 (also known as TP53 in humans and Trp53 in mice) short-interfering RNA (siRNA) was recently shown to promote human iPS cell generation, but the specificity and mechanisms remain to be determined. Here we report that up to 10% of transduced mouse embryonic fibroblasts lacking p53 became iPS cells, even without the Myc retrovirus. The p53 deletion also promoted the induction of integration-free mouse iPS cells with plasmid transfection. Furthermore, in the p53-null background, iPS cells were generated from terminally differentiated T lymphocytes. The suppression of p53 also increased the efficiency of human iPS cell generation. DNA microarray analyses identified 34 p53-regulated genes that are common in mouse and human fibroblasts. Functional analyses of these genes demonstrate that the p53-p21 pathway serves as a barrier not only in tumorigenicity, but also in iPS cell generation.

Hwang, W. S., Y. J. Ryu, et al. (2004). "Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst." Science **303**(5664): 1669-74.

Somatic cell nuclear transfer (SCNT) technology has recently been used to generate animals with a common genetic composition. In this study, we report the derivation of a pluripotent embryonic stem (ES) cell line (SCNT-hES-1) from a cloned human blastocyst. The SCNT-hES-1 cells displayed typical ES cell morphology and cell surface markers and were capable of differentiating into embryoid bodies in vitro and of forming teratomas in vivo containing cell derivatives from all three embryonic germ layers in severe combined immunodeficient mice. After continuous proliferation for more than 70 passages, SCNT-hES-1 cells maintained normal karyotypes and were genetically identical to the somatic nuclear donor cells. Although we cannot completely exclude the possibility that the cells had a parthenogenetic origin, imprinting analyses support a SCNT origin of the derived human ES cells.

Ito, H., Y. Takeuchi, et al. (2004). "Local irradiation enhances congenic donor pluripotent hematopoietic stem cell engraftment similarly in irradiated and nonirradiated sites." Blood **103**(5): 1949-54.

Long-term multilineage chimerism is achieved in CD45 congenic mice receiving high bone marrow doses with or without mediastinal irradiation (MI). Increased donor chimerism results in MI-treated compared with nonirradiated animals, suggesting that MI makes "space" for engraftment of donor pluripotent hematopoietic stem cells (PHSCs). We have now examined whether space is systemic or whether increased engraftment of donor marrow in locally irradiated mice is confined to the irradiated bones. While increased donor chimerism was observed in irradiated bones compared with nonirradiated bones of MI-treated animals 4 weeks following bone marrow transplantation (BMT), these differences were minimal by 40 weeks. MI-treated chimeras contained more adoptively transferable donor PHSCs in the marrow of both irradiated and distant bones compared with non-MI-treated chimeras.

Similar proportions of donor PHSCs were present in irradiated and nonirradiated bones of locally irradiated mice at both 4 and 40 weeks. Irradiated bones contained more donor short-term repopulating cells than distant bones at 4 weeks, but not 40 weeks, after BMT. Our study suggests that local proliferation of donor PHSCs in mice receiving local irradiation rapidly leads to a systemic increase in donor PHSC engraftment.

Ji, Z. and B. Tian (2009). "Reprogramming of 3' untranslated regions of mRNAs by alternative polyadenylation in generation of pluripotent stem cells from different cell types." PLoS One **4**(12): e8419.

BACKGROUND: The 3' untranslated regions (3'UTRs) of mRNAs contain cis elements involved in post-transcriptional regulation of gene expression. Over half of all mammalian genes contain multiple polyadenylation sites that lead to different 3'UTRs for a gene. Studies have shown that the alternative polyadenylation (APA) pattern varies across tissues, and is dynamically regulated in proliferating or differentiating cells. Generation of induced pluripotent stem (iPS) cells, in which differentiated cells are reprogrammed to an embryonic stem (ES) cell-like state, has been intensively studied in recent years. However, it is not known how 3'UTRs are regulated during cell reprogramming. METHODS/MAIN FINDINGS: Using a computational method that robustly examines APA across DNA microarray data sets, we analyzed 3'UTR dynamics in generation of iPS cells from different cell types. We found that 3'UTRs shorten during reprogramming of somatic cells, the extent of which depends on the type of source cell. By contrast, reprogramming of spermatogonial cells involves 3'UTR lengthening. The alternative polyadenylation sites that are highly responsive to change of cell state in generation of iPS cells are also highly regulated during embryonic development in opposite directions. Compared with other sites, they are more conserved, can lead to longer alternative 3'UTRs, and are associated with more cis elements for polyadenylation. Consistently, reprogramming of somatic cells and germ cells involves significant upregulation and downregulation, respectively, of mRNAs encoding polyadenylation factors, and RNA processing is one of the most significantly regulated biological processes during cell reprogramming. Furthermore, genes containing target sites of ES cell-specific microRNAs (miRNAs) in different portions of 3'UTR are distinctively regulated during cell reprogramming, suggesting impact of APA on miRNA targeting. CONCLUSIONS/SIGNIFICANCE: Taken together, these findings indicate that reprogramming of 3'UTRs by APA, which result from regulation of both general polyadenylation activity and cell type-specific factors and can reset post-transcriptional gene regulatory programs in the cell, is an integral part of iPS cell generation, and the APA pattern can be a good biomarker for cell type and state, useful for sample classification. The results also suggest that perturbation of the mRNA polyadenylation machinery or RNA processing activity may facilitate generation of iPS cells.

Kakuda, H., T. Sato, et al. (1996). "A novel human leukaemic cell line, CTS, has a t(6;11) chromosomal translocation and characteristics of pluripotent stem cells." Br J Haematol **95**(2): 306-18.

A novel human leukaemic cell line, designated CTS, was established from the peripheral blood of a 13-year-old girl suffering from acute myeloblastic leukaemia (AML) in relapse. CTS cells expressed CD7, CD13, CD33, CD34 and HLA-DR antigens, and showed ultrastructural myeloperoxidase activity. In addition, CTS cells showed DNA rearrangements of the immunoglobulin heavy chain gene and the light kappa chain gene, and deletions of the T-cell receptor delta 1 gene. Cytogenetic analysis revealed a human female diploid karyotype with a t(6;11)(q27;q23) chromosomal translocation. Molecular studies demonstrated a DNA rearrangement of the MLL gene, the expression of a truncated 11.0 kb MLL mRNA and the detection of the MLL/AF-6 fusion transcript in CTS cells. To our knowledge, this cell line is the first report of a human leukaemic cell line with a t(6;11) chromosomal translocation. CTS cells showed no significant proliferative response to the cytokines, IL-2, IL-3, IL-6, IL-11, GM-CSF, G-CSF, EPO, SCF, but were induced to differentiate to the T-cell, B-cell, erythroid or megakaryocytic lineage in the presence of particular cytokines. This CTS cell line may provide a useful tool in the study of the oncogenesis of mixed lineage leukaemia with 11q23 abnormalities and for the analysis of growth and differentiation of pluripotent stem cells.

Kerr, C. L., C. M. Hill, et al. (2008). "Expression of pluripotent stem cell markers in the human fetal ovary." Hum Reprod **23**(3): 589-99.

BACKGROUND: Human primordial germ cells (PGCs) can give rise to pluripotent stem cells such as embryonal carcinoma cells (ECCs) and embryonic germ cells (EGCs). METHODS: In order to determine whether PGCs express markers associated with pluripotency in EGCs and ECCs, the following study cross examines the expression patterns of multiple pluripotent markers in the human fetal ovary, 5.5-15 weeks post-fertilizaton (pF) and relates this expression with the ability to derive

pluripotent EGCs in vitro. RESULTS: Specific subpopulations were identified which included OCT4(+)/Nanog(+)/cKIT(+)/VASA(+) PGCs and oogonia. Interestingly, these cells also expressed SSEA1 and alkaline phosphatase (AP) and SSEA4 expression occurred throughout the entire gonad. Isolation of SSEA1(+) cells from the gonad resulted in AP(+) EGC colony formation. The number of OCT4(+) or Nanog(+) expressing cells peaked by week 8 and then diminished after week 9 pF, as oogonia enter meiosis. In addition, the efficiency of EGC derivation was associated with the number of OCT4(+) cells. TRA-1-60 and TRA-1-81 were only detected in the lining of the mesonephric ducts and occasionally in the gonad. CONCLUSIONS: These results demonstrate that PGCs, a unipotent cell, express most, but not all, of the markers associated with pluripotent cells in the human fetal ovary.

Kerr, C. L., C. M. Hill, et al. (2008). "Expression of pluripotent stem cell markers in the human fetal testis." Stem Cells **26**(2): 412-21.

Human primordial germ cells (PGCs) have proven to be a source of pluripotent stem cells called embryonic germ cells (EGCs). However, the developmental potency of these cells in the fetal gonad still remains elusive. Thus, this study provides a comprehensive analysis of pluripotent and germ cell marker expression in human fetal testis 7-15 weeks postfertilization (pF) and compares this expression to their ability to derive EGCs. Although the majority of germ cells expressed stem cell markers stage-specific embryonic antigen (SSEA) 1, SSEA4, EMA-1, and alkaline phosphatase, only a small percentage of those (<1%) expressed OCT4, CKIT, and NANOG. Specifically, the number of OCT4(+)/CKIT(+)/NANOG(+) cells significantly increased in the developing cords during weeks 7-9, followed by a gradual decline into week 15 pF. By week 15 pF, the remaining OCT4(+)/CKIT(+)/NANOG(+) cells were found in the cords surrounding the periphery of the testis, and the predominant germ cells, CKIT(+) cells, no longer expressed OCT4 or NANOG. Based on morphology and early germ cell marker expression, including VASA, PUM2, and DAZL, we suggest these cells are mitotically active gonocytes or prespermatogonia. Importantly, the number of OCT4(+) cells correlated with an increase in the number of EGC colonies derived in culture. Interestingly, two pluripotent markers, Tra-1-60 and Tra-1-81, although highly expressed in EGCs, were not expressed by PGCs in the gonad. Together, these results suggest that PGCs maintain expression of pluripotent stem cell markers during and after sexual differentiation of the gonad, albeit in very low numbers.

Kirshenbaum, A. S., J. P. Goff, et al. (1992). "Effect of IL-3 and stem cell factor on the appearance of human basophils and mast cells from CD34+ pluripotent progenitor cells." J Immunol **148**(3): 772-7.

Hemopoietic stem cell factor (SCF), which is the ligand for the proto-oncogene c-kit receptor (allelic with W locus) and the product of Sl locus of the mouse, has recently been cloned. The human homologue has also been cloned, and recombinant protein (human rSCF) expressed and purified to homogeneity. To determine the effect of human rSCF in the presence or absence of human rIL-3 on human bone marrow-derived mast cells and basophils, human CD34+ pluripotent progenitor cells, highly enriched (greater than 99%) from bone marrow mononuclear cells, were cultured over agarose surfaces (interphase cultures) in the presence of human rIL-3, human rIL-3 and increasing concentrations of human rSCF, or human rSCF alone. Over 3 to 4 wk, human rSCF acted synergistically with human rIL-3 at all concentrations, producing a three- to fivefold increase in total, mast cell, and basophil numbers over human rIL-3 alone when used at 100 ng/ml. The percentage of cell types in the human rIL-3 and human rIL-3 plus human rSCF cultures, however, remained the same, with basophils constituting 18 to 35% of the final cultured cells, and mast cells 3% or less of the final cell number. In the presence of human rSCF alone, the combined total percentage of mast cells and basophils was 0 to 1.0%, the majority of cells being macrophages. Mast cells cultured in human rIL-3 plus human rSCF, but not human rIL-3 alone, were berberine sulfate positive, suggesting the presence of heparin proteoglycans within granules. Electron microscopic examination of cultures supplemented with human rIL-3 and rSCF, but not human rIL-3 alone, revealed that after 3 wk in culture, mast cell granules contained tryptase and exhibited scroll, reticular, and homogeneous patterns as seen previously in CD34+/3T3 fibroblast cocultures. Thus, CD34+ cells cultured in the presence of both human rIL-3 and rSCF give rise to cultures containing increased numbers of basophils and mast cells, with the mast cells by ultrastructural studies showing evidence of maturation although the percentages of basophils and mast cells arising in these cultures remained unchanged.

Kodama, H. A., Y. Amagai, et al. (1982). "A new preadipose cell line derived from newborn mouse calvaria can promote the proliferation of pluripotent hemopoietic stem cells in vitro." J Cell Physiol **112**(1): 89-95.

A clonal preadipose cell line MC3T3-G2/PA6, established from newborn mouse calvaria,

responds to glucocorticoids and converts to adipose cells in a fashion similar to bone marrow preadipocytes. We investigated the effect of the cells on in vitro hemopoiesis of mouse bone marrow cells by cocultivation. When bone marrow cells were inoculated into confluent cultures of MC3T3-G2/PA6 cells (10(4)-10(6) cells/25-cm2 flask), the number of hemopoietic stem cells (CFU-S) significantly increased during 7-day cultivation in proportion to inoculum size. Under these conditions, active replication of CFU-S was maintained for several weeks until MC3T3-G2/PA6 cell layers detached from the substratum. This capacity of the MC3T3-G2/PA6 line was unique because other established cell lines, including the MTF preadipose line, failed to support CFU-S growth. When bone marrow cells were not allowed to contact the MC3T3-G2/PA6 cell layer, only a small number of CFU-S survived for 7 days. Moreover, MC3T3-G2/PA6 cell-conditioned medium did not show any growth-promoting activity for CFU-S. These results indicate that the MC3T3-G2/PA6 cell line has the ability to promote the proliferation of CFU-S through a short range cell-to-cell interaction by providing an in vitro microenvironment probably similar to that for in vivo hemopoiesis.

Kogler, G., S. Sensken, et al. (2004). "A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential." J Exp Med **200**(2): 123-35.

Here a new, intrinsically pluripotent, CD45-negative population from human cord blood, termed unrestricted somatic stem cells (USSCs) is described. This rare population grows adherently and can be expanded to 10(15) cells without losing pluripotency. In vitro USSCs showed homogeneous differentiation into osteoblasts, chondroblasts, adipocytes, and hematopoietic and neural cells including astrocytes and neurons that express neurofilament, sodium channel protein, and various neurotransmitter phenotypes. Stereotactic implantation of USSCs into intact adult rat brain revealed that human Tau-positive cells persisted for up to 3 mo and showed migratory activity and a typical neuron-like morphology. In vivo differentiation of USSCs along mesodermal and endodermal pathways was demonstrated in animal models. Bony reconstitution was observed after transplantation of USSC-loaded calcium phosphate cylinders in nude rat femurs. Chondrogenesis occurred after transplanting cell-loaded gelfoam sponges into nude mice. Transplantation of USSCs in a noninjury model, the preimmune fetal sheep, resulted in up to 5% human hematopoietic engraftment. More than 20% albumin-producing human parenchymal hepatic cells with absence of cell fusion and substantial numbers of human cardiomyocytes in both atria and ventricles of the sheep heart were detected many months after USSC transplantation. No tumor formation was observed in any of these animals.

Kossack, N., J. Meneses, et al. (2009). "Isolation and characterization of pluripotent human spermatogonial stem cell-derived cells." Stem Cells **27**(1): 138-49.

Several reports have documented the derivation of pluripotent cells (multipotent germline stem cells) from spermatogonial stem cells obtained from the adult mouse testis. These spermatogonia-derived stem cells express embryonic stem cell markers and differentiate to the three primary germ layers, as well as the germline. Data indicate that derivation may involve reprogramming of endogenous spermatogonia in culture. Here, we report the derivation of human multipotent germline stem cells (hMGSCs) from a testis biopsy. The cells express distinct markers of pluripotency, form embryoid bodies that contain derivatives of all three germ layers, maintain a normal XY karyotype, are hypomethylated at the H19 locus, and express high levels of telomerase. Teratoma assays indicate the presence of human cells 8 weeks post-transplantation but limited teratoma formation. Thus, these data suggest the potential to derive pluripotent cells from human testis biopsies but indicate a need for novel strategies to optimize hMGSC culture conditions and reprogramming.

Labat, M. L., M. Pouchelet, et al. (2001). "Regulation by phagic T-lymphocytes of a (pluripotent?) organ stem cell present in adult human blood. A beneficial exception to self-tolerance." Biomed Pharmacother **55**(2): 79-90.

Stem cells isolated from adult human blood are able to give rise to several different kinds of cell types such as mesenchymal cells, including striated muscle cells, hepatocytes, and endothelial-cells. Because independently studied by authors whose interests focused on particular tissue types, these stem cells have been described as different. However, they might well represent one unique population of pluripotent stem cells in homeostatic equilibrium with the 'reserve' stem cells buried in organs. In the blood, these stem cells have a monocytic phenotype. In in vitro culture, once they have adhered, they spontaneously differentiate into diverse types of cells reminiscent of embryonic stem cells in culture. Normally, they are almost quiescent cells. But under precise circumstances such as wound-healing, they may proliferate and migrate to the right organ to give rise there to the right type of cells, in order to participate in the repair process. Indeed, such a powerful stem cell needs to be tightly controlled. We illustrate here, by time-lapse videocinematography,

how a special subpopulation of T-lymphocytes, for which we coined the name 'phagic T-lymphocytes' (PTLs), destroys these stem cells as soon as they differentiate in vitro, i.e., without the purpose of a repair. These stem cells express constitutively HLA-DR molecules and therefore can act as antigen-presenting cells able to activate phagic T-lymphocytes. The targets of these activated phagic T-lymphocytes are the differentiated stem cell themselves. Phagic T-lymphocytes are attracted by the stem cells, circulate around them, then penetrate and circulate inside them until the latter 'explode'. This mechanism of destruction by phagic T-lymphocytes is unique and seems to be normally restricted to stem cells. It represents a beneficial exception in self-tolerance since it avoids the accumulation of these stem cells out of healing purposes. Interestingly, in disorders such as fibrosis and/or some malignant proliferations, these stem cells proliferate, escape destruction by phagic T-lymphocytes and, as a consequence, accumulate, giving rise to a 'tissue' when cultured in vitro.

Lenfant, M., J. Wdzieczak-Bakala, et al. (1989). "Inhibitor of hematopoietic pluripotent stem cell proliferation: purification and determination of its structure." Proc Natl Acad Sci U S A **86**(3): 779-82.

We report here a five-step purification procedure that led to the isolation from fetal calf bone marrow extract of a tetrapeptide, Ac-Ser-Asp-Lys-Pro (Mr 487), exerting a high inhibitory activity on the proliferation of hematopoietic pluripotent stem cells [defined here as spleen colony-forming units (CFU-S)]. The structure of this molecule was established from amino acid analysis, fast atom bombardment mass spectrometry, and 1H nuclear magnetic resonance spectral data. This structure was confirmed by comparison with the corresponding synthetic molecule, which presents identical physiochemical characteristics and biological properties. Natural and synthetic peptides administered to mice (at a dose of 100 ng per mouse) after one injection of cytosine arabinonucleoside prevent CFU-S recruitment into DNA synthesis.

Li, C., Y. Yang, et al. (2009). "Derivation and transcriptional profiling analysis of pluripotent stem cell lines from rat blastocysts." Cell Res **19**(2): 173-86.

Embryonic stem (ES) cells are derived from blastocyst-stage embryos. Their unique properties of self-renewal and pluripotency make them an attractive tool for basic research and a potential cell resource for therapy. ES cells of mouse and human have been successfully generated and applied in a wide range of research. However, no genuine ES cell lines have been obtained from rat to date. In this study, we identified pluripotent cells in early rat embryos using specific antibodies against markers of pluripotent stem cells. Subsequently, by modifying the culture medium for rat blastocysts, we derived pluripotent rat ES-like cell lines, which expressed pluripotency markers and formed embryoid bodies (EBs) in vitro. Importantly, these rat ES-like cells were able to produce teratomas. Both EBs and teratomas contained tissues from all three embryonic germ layers. In addition, from the rat ES-like cells, we derived a rat primitive endoderm (PrE) cell line. Furthermore, we conducted transcriptional profiling of the rat ES-like cells and identified the unique molecular signature of the rat pluripotent stem cells. Our analysis demonstrates that multiple signaling pathways, including the BMP, Activin and mTOR pathways, may be involved in keeping the rat ES-like cells in an undifferentiated state. The cell lines and information obtained in this study will accelerate our understanding of the molecular regulation underlying pluripotency and guide us in the appropriate manipulation of ES cells from a particular species.

Li, C., H. Yu, et al. (2009). "Germline-competent mouse-induced pluripotent stem cell lines generated on human fibroblasts without exogenous leukemia inhibitory factor." PLoS One **4**(8): e6724.

Induced pluripotent stem (iPS) cells have attracted enormous attention due to their vast potential in regenerative medicine, pharmaceutical screening and basic research. Most prior established iPS cell lines were derived and maintained on mouse embryonic fibroblast (MEF) cells supplemented with exogenous leukemia inhibitory factor (LIF). Drawbacks of MEF cells impede optimization as well as dissection of reprogramming events and limit the usage of iPS cell derivatives in therapeutic applications. In this study, we develop a reproducible protocol for efficient reprogramming mouse neural progenitor cells (NPCs) on human foreskin fibroblast (HFF) cells via retroviral transfer of human transcriptional factors OCT4/SOX2/KLF4/C-MYC. Two independent iPS cell lines are derived without exogenous LIF. They display typical undifferentiated morphology and express pluripotency markers Oct4 and Sox2. Transgenes are inactivated and the endogenous Oct4 promoter is completely demethylated in the established iPS cell lines, indicating a fully reprogrammed state. Moreover, the iPS cells can spontaneously differentiate or be induced into various cell types of three embryonic germ layers in vitro and in vivo when they are injected into immunodeficient mice for teratoma formation. Importantly, iPS cells extensively integrate with various host tissues and contribute to the germline

when injected into the blastocysts. Interestingly, these two iPS cell lines, while both pluripotent, exhibit distinctive differentiation tendencies towards different lineages. Taken together, the data describe the first genuine mouse iPS cell lines generated on human feeder cells without exogenous LIF, providing a reliable tool for understanding the molecular mechanisms of nuclear reprogramming.

Lu, M., M. D. Kardel, et al. (2009). "Enhanced generation of hematopoietic cells from human hepatocarcinoma cell-stimulated human embryonic and induced pluripotent stem cells." Exp Hematol **37**(8): 924-36.

OBJECTIVE: Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) constitute unique sources of pluripotent cells, although the molecular mechanisms involved in their differentiation into specific lineages are just beginning to be defined. Here we evaluated the ability of MEDII (medium conditioned by HepG2 cells, a human hepatocarcinoma cell line) to selectively enhance generation of mesodermal derivatives, including hematopoietic cells, from hESCs and hiPSCs. MATERIALS AND METHODS: Test cells were exposed to MEDII prior to being placed in conditions that promote embryoid body (EB) formation. Hematopoietic activity was measured by clonogenic assays, flow cytometry, quantitative real-time polymerase chain reaction of specific transcript complementary DNAs and the ability of cells to repopulate sublethally irradiated nonobese diabetic/severe combined immunodeficient interleukin-2 receptor gamma-chain-null mice for almost 1 year. RESULTS: Exposure of both hESCs and hiPSCs to MEDII induced a rapid and preferential differentiation of hESCs into mesodermal elements. Subsequently produced EBs showed a further enhanced expression of transcripts characteristic of multiple mesodermal lineages, and a concurrent decrease in endodermal and ectodermal cell transcripts. Frequency of all types of clonogenic hematopoietic progenitors in subsequently derived EBs was also increased. In vivo assays of MEDII-treated hESC-derived EBs also showed they contained cells able to undertake low-level but longterm multilineage repopulation of primary and secondary nonobese diabetic/severe combined immunodeficient interleukin-2 receptor gamma-chain-null mice. CONCLUSIONS: MEDII treatment of hESCs and hiPSCs alike selectively enhances their differentiation into mesodermal cells and allows subsequent generation of detectable levels of hematopoietic progenitors with in vitro and in vivo differentiating activity.

Marion, R. M., K. Strati, et al. (2009). "Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells." Cell Stem Cell **4**(2): 141-54.

Telomere shortening is associated with organismal aging. iPS cells have been recently derived from old patients; however, it is not known whether telomere chromatin acquires the same characteristics as in ES cells. We show here that telomeres are elongated in iPS cells compared to the parental differentiated cells both when using four (Oct3/4, Sox2, Klf4, cMyc) or three (Oct3/4, Sox2, Klf4) reprogramming factors and both from young and aged individuals. We demonstrate genetically that, during reprogramming, telomere elongation is usually mediated by telomerase and that iPS telomeres acquire the epigenetic marks of ES cells, including a low density of trimethylated histones H3K9 and H4K20 and increased abundance of telomere transcripts. Finally, reprogramming efficiency of cells derived from increasing generations of telomerase-deficient mice shows a dramatic decrease in iPS cell efficiency, a defect that is restored by telomerase reintroduction. Together, these results highlight the importance of telomere biology for iPS cell generation and functionality.

Martin, G. R. (1981). "Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells." Proc Natl Acad Sci U S A **78**(12): 7634-8.

This report describes the establishment directly from normal preimplantation mouse embryos of a cell line that forms teratocarcinomas when injected into mice. The pluripotency of these embryonic stem cells was demonstrated conclusively by the observation that subclonal cultures, derived from isolated single cells, can differentiate into a wide variety of cell types. Such embryonic stem cells were isolated from inner cell masses of late blastocysts cultured in medium conditioned by an established teratocarcinoma stem cell line. This suggests that such conditioned medium might contain a growth factor that stimulates the proliferation or inhibits the differentiation of normal pluripotent embryonic cells, or both. This method of obtaining embryonic stem cells makes feasible the isolation of pluripotent cells lines from various types of noninbred embryo, including those carrying mutant genes. The availability of such cell lines should made possible new approaches to the study of early mammalian development.

Moore, J. C., J. Fu, et al. (2008). "Distinct cardiogenic preferences of two human embryonic stem cell (hESC) lines are imprinted in their proteomes in the

pluripotent state." Biochem Biophys Res Commun **372**(4): 553-8.

Although both the H1 and HES2 human embryonic stem cell lines (NIH codes: WA01 and ES02, respectively) are capable of forming all three germ layers and their derivatives, various lines of evidence including the need to use different protocols to induce cardiac differentiation hint that they have distinct preferences to become chamber-specific heart cells. However, a direct systematic comparison has not been reported. Here we electrophysiologically demonstrated that the distributions of ventricular-, atrial- and pacemaker-like derivatives were indeed different (ratios=39:61:0 and 64:33:3 for H1 and HES2, respectively). Based on these results, we hypothesized the differences in their cardiogenic potentials are imprinted in the proteomes of undifferentiated H1 and HES2. Using multiplexing, high-resolution 2-D Differential In Gel Electrophoresis (DIGE) to minimize gel-to-gel variations that are common in conventional 2-D gels, a total of 2000 individual protein spots were separated. Of which, 55 were >2-fold differentially expressed in H1 and HES2 ($p<0.05$) and identified by mass spectrometery. Bioinformatic analysis of these protein differences further revealed candidate pathways that contribute to the H1 and HES2 phenotypes. We conclude that H1 and HES2 have predetermined preferences to become ventricular, atrial, and pacemaker cells due to discrete differences in their proteomes. These results improve our basic understanding of hESCs and may lead to mechanism-based methods for their directed cardiac differentiation into chamber-specific cardiomyocytes.

Munsie, M. J., A. E. Michalska, et al. (2000). "Isolation of pluripotent embryonic stem cells from reprogrammed adult mouse somatic cell nuclei." Curr Biol **10**(16): 989-92.

Pluripotent human stem cells isolated from early embryos represent a potentially unlimited source of many different cell types for cell-based gene and tissue therapies [1-3]. Nevertheless, if the full potential of cell lines derived from donor embryos is to be realised, the problem of donor-recipient tissue matching needs to be overcome. One approach, which avoids the problem of transplant rejection, would be to establish stem cell lines from the patient's own cells through therapeutic cloning [3,4]. Recent studies have shown that it is possible to transfer the nucleus from an adult somatic cell to an unfertilised oocyte that is devoid of maternal chromosomes, and achieve embryonic development under the control of the transferred nucleus [5-7]. Stem cells isolated from such a cloned embryo would be genetically identical to the patient and pose no risk of immune rejection. Here, we report the isolation of pluripotent murine stem cells from reprogrammed adult somatic cell nuclei. Embryos were generated by direct injection of mechanically isolated cumulus cell nuclei into mature oocytes. Embryonic stem (ES) cells isolated from cumulus-cell-derived blastocysts displayed the characteristic morphology and marker expression of conventional ES cells and underwent extensive differentiation into all three embryonic germ layers (endoderm, mesoderm and ectoderm) in tumours and in chimaeric foetuses and pups. The ES cells were also shown to differentiate readily into neurons and muscle in culture. This study shows that pluripotent stem cells can be derived from nuclei of terminally differentiated adult somatic cells and offers a model system for the development of therapies that rely on autologous, human pluripotent stem cells.

Nakayama, M. (2009). "Cell Therapy Using Induced Pluripotent Stem (iPS) Cells Meets Next-Next Generation DNA Sequencing Technology." Curr Genomics **10**(5): 303-5.

The recent development of induced pluripotent stem (iPS) cell technology brings cell and gene therapies to patients one large step closer to reality. Technical improvements in various research fields sometimes come together fortuitously, leading to approaches to treating disease. If iPS cell technology continues to progress smoothly as expected and is actually applied to patients, the next logical step to ensuring the success of iPS cell therapy is to make use of next-next generation DNA sequencing technology and bioinformatics of recipient genomes. Before a patient-derived iPS cell colony is used for clinical therapy in a patient, the colony should undergo whole-genome DNA sequencing, thus avoiding risks associated with spontaneously mutagenized iPS cells. Researchers participating in the Human Genome Project need to take full advantage of both technologies-iPS cell technology and DNA sequencing-as doing so will help us achieve the original long-term goal of the project: developing therapies that will benefit human health.

Nijhof, W. and P. K. Wierenga (1985). "Characterization of a cycling murine pluripotent stem cell population." Eur J Cell Biol **39**(1): 136-41.

In the present report we describe the properties of pluripotent stem cells isolated with elutriation and Percoll density gradient centrifugation techniques from spleens of recovering thiamphenicol pretreated anemic mice. Cells with a diameter between 7.2 and 8.4 microns and sedimenting at a density of 1.065 g/ml had a high capacity to repopulate lethally irradiated mice with all the hemopoietic precursor cells. The spleen colonies formed on day 8 and day 12

after inoculation in irradiated recipients showed no morphological differences in mixed and lineage-specific composition. Day 12 colonies were much larger than day 8 colonies. Pluripotent stem cells isolated from regenerating spleens were very susceptible to 3'-thymidine; 65% of the cells were killed. Only 2% kill was found in stem cells present in vivo 4 days earlier in the bone marrow. The purified stem cells were probably all in cycle and possibly partly synchronized.

Orlic, D., R. Fischer, et al. (1993). "Purification and characterization of heterogeneous pluripotent hematopoietic stem cell populations expressing high levels of c-kit receptor." Blood **82**(3): 762-70.

Mouse pluripotent hematopoietic stem cells (PHSC) were fractionated based on size and density using counterflow centrifugal elutriation (CCE). These heterogeneous PHSC populations were further enriched by subtraction of cells with lineage-specific markers (Lin-) followed by positive sorting for c-kit expression. The cells were characterized for their functional and biochemical properties. We defined a subpopulation of c-kit-positive cells that expressed high numbers of c-kit receptors (c-kitBR). One hundred c-kitBR cells from either low- or higher-density fractions were sufficient to repopulate the lymphohematopoietic system in WBB6F1-W/Wv (W/Wv) recipients, whereas no PHSC were found in cells with low (c-kitDULL) or no (c-kitNEG) c-kit expression. Lin- c-kitBR cells were separated into RhoDULL and RhoBR subsets based on their ability to efflux rhodamine 123 (Rho). The PHSC were concentrated in Lin- c-kitBR RhoDULL cells and the number of Lin- c-kitBR RhoBR cells correlated directly with the number of day 12 colony-forming unit-spleen (CFU-S12) in each fraction. We were not able to enrich further for PHSC using monoclonal antibodies to the cell-surface markers AA4.1 or CD4, which have been used by others to isolate PHSC. The small, low-density Lin- c-kitBR subset contained PHSC and few CFU-S12. This enabled us to assay PHSC for expression of the flk-2 gene, which encodes a tyrosine kinase receptor present on fetal liver PHSC. Purified RNA from the low-density Lin- c-kitBR subset did not contain flk-2 mRNA. We suggest that AA4.1, CD4 and flk-2 are expressed as stage-specific markers on PHSC in cell cycle.

Parameswaran, V., R. Shukla, et al. (2007). "Development of a pluripotent ES-like cell line from Asian sea bass (Lates calcarifer)--an oviparous stem cell line mimicking viviparous ES cells." Mar Biotechnol (NY) **9**(6): 766-75.

We report a pluripotent embryonic stem cell-like cell line designated as SBES from blastula stage embryos of Asian sea bass (Lates calcarifer), which is an economically important cultivable and edible marine fish species in India. The SBES cells were cultured at 28 degrees C in Leibovitz L-15 medium supplemented with 20% fetal bovine serum without a feeder layer. The ES-like cells were round or polygonal and grew exponentially in culture. The SBES cells exhibited an intense alkaline phosphatase activity and expression of transcription factor Oct 4. The undifferentiated state of these cells was maintained at low seeding densities and the cells formed embryoid bodies when seeded in bacteriological plates. On treatment with all-trans retinoic acid, these cells differentiated into neuron-like cells, muscle cells, and beating cardiomyocytes, indicating their pluripotency. This embryonic ES-like cell line derived from an oviparous fish blastula conserved several peculiar features of viviparous mammalian embryonic stem cell lines. The present study highlights the importance and potential of piscine ES-like cell line for stem cell research without evoking ethical issues and invasive interventions sparing mammalian embryos.

Peerani, R., K. Onishi, et al. (2009). "Manipulation of signaling thresholds in "engineered stem cell niches" identifies design criteria for pluripotent stem cell screens." PLoS One **4**(7): e6438.

In vivo, stem cell fate is regulated by local microenvironmental parameters. Governing parameters in this stem cell niche include soluble factors, extra-cellular matrix, and cell-cell interactions. The complexity of this in vivo niche limits analyses into how individual niche parameters regulate stem cell fate. Herein we use mouse embryonic stem cells (mESC) and micro-contact printing (microCP) to investigate how niche size controls endogenous signaling thresholds. microCP is used to restrict colony diameter, separation, and degree of clustering. We show, for the first time, spatial control over the activation of the Janus kinase/signal transducer and activator of transcription pathway (Jak-Stat). The functional consequences of this niche-size-dependent signaling control are confirmed by demonstrating that direct and indirect transcriptional targets of Stat3, including members of the Jak-Stat pathway and pluripotency-associated genes, are regulated by colony size. Modeling results and empirical observations demonstrate that colonies less than 100 microm in diameter are too small to maximize endogenous Stat3 activation and that colonies separated by more than 400 microm can be considered independent from each other. These results define parameter boundaries for the use of ESCs in screening studies, demonstrate the importance of context in stem cell responsiveness to exogenous cues,

and suggest that niche size is an important parameter in stem cell fate control.

Pradelles, P., Y. Frobert, et al. (1990). "Negative regulator of pluripotent hematopoietic stem cell proliferation in human white blood cells and plasma as analysed by enzyme immunoassay." Biochem Biophys Res Commun **170**(3): 986-93.

This paper describes the analysis, by a highly sensitive and specific enzyme immunoassay (EIA), of AcSDKP, a tetrapeptide recently isolated from fetal calf bone marrow and subsequently purified and identified which substantially inhibits entry into cycle of hematopoietic pluripotent stem cells (CFU-S). This molecule has a marked protective effect in mice during anticancer chemotherapy with phase-specific drugs and plays an essential role in maintaining CFU-S out of cycle in normal mice. Using acetylcholinesterase-AcSDKP conjugate as tracer, rabbit specific antiserum and 96-well microtiter plates coated with a mouse monoclonal anti-rabbit IgG antibody, this EIA allows detection of AcSDKP at 15 fmol levels with a coefficient of variation less than 10% in the 50-500 fmol range. When combined with high-performance liquid chromatography, this assay clearly reveals the presence of this peptide in normal human white blood cells whereas in supernatant from cultured lymphocytes and in plasma the immunoreactive material is distinct from standard AcSDKP.

Prelle, K., N. Zink, et al. (2002). "Pluripotent stem cells--model of embryonic development, tool for gene targeting, and basis of cell therapy." Anat Histol Embryol **31**(3): 169-86.

Embryonic stem (ES) cells are pluripotent cell lines with the capacity of self-renewal and a broad differentiation plasticity. They are derived from pre-implantation embryos and can be propagated as a homogeneous, uncommitted cell population for an almost unlimited period of time without losing their pluripotency and their stable karyotype. Murine ES cells are able to reintegrate fully into embryogenesis when returned into an early embryo, even after extensive genetic manipulation. In the resulting chimeric offspring produced by blastocyst injection or morula aggregation, ES cell descendants are represented among all cell types, including functional gametes. Therefore, mouse ES cells represent an important tool for genetic engineering, in particular via homologous recombination, to introduce gene knock-outs and other precise genomic modifications into the mouse germ line. Because of these properties ES cell technology is of high interest for other model organisms and for livestock species like cattle and pigs. However, in spite of tremendous research activities, no proven ES cells colonizing the germ line have yet been established for vertebrate species other than the mouse (Evans and Kaufman, 1981; Martin, 1981) and chicken (Pain et al., 1996). The in vitro differentiation capacity of ES cells provides unique opportunities for experimental analysis of gene regulation and function during cell commitment and differentiation in early embryogenesis. Recently, pluripotent stem cells were established from human embryos (Thomson et al., 1998) and early fetuses (Shamblott et al., 1998), opening new scenarios both for research in human developmental biology and for medical applications, i.e. cell replacement strategies. At about the same time, research activities focused on characteristics and differentiation potential of somatic stem cells, unravelling an unexpected plasticity of these cell types. Somatic stem cells are found in differentiated tissues and can renew themselves in addition to generating the specialized cell types of the tissue from which they originate. Additional to discoveries of somatic stem cells in tissues that were previously not thought to contain these kinds of cells, they also appear to be capable of developing into cell types of other tissues, but have a reduced differentiation potential as compared to embryo-derived stem cells. Therefore, somatic stem cells are referred to as multipotent rather than pluripotent. This review summarizes characteristics of pluripotent stem cells in the mouse and in selected livestock species, explains their use for genetic engineering and basic research on embryonic development, and evaluates their potential for cell therapy as compared to somatic stem cells.

Qin, J., X. Guo, et al. (2009). "Cluster characterization of mouse embryonic stem cell-derived pluripotent embryoid bodies in four distinct developmental stages." Biologicals **37**(4): 235-44.

The formation of embryoid bodies (EBs) is the principal step in the differentiation of embryonic stem (ES) cells. In this study, the morphological characteristics and gene expression patterns of EBs related to the sequential stages of embryonic development were well defined in four distinct developmental groups over 112 days of culture: early-stage EBs groups (1-7 days of differentiation), mid-stage EBs groups (9-15 days of differentiation), maturing EBs groups (17-45 days of differentiation) and matured EBs groups (50 days of differentiation). We first determined definite histological location of apoptosis within EBs and the sequential expression of molecular markers representing stem cells (Oct4, SSEA-1, Sox-2 and AKP), germ cells (Fragilis, Dazl, c-kit, StellaR, Mvh and Stra8), ectoderm (Neurod, Nestin and Neurofilament), mesoderm (Gata-1, Flk-1 and Hbb) and endoderm (AFP and Transthyretin). Our

results revealed that developing EBs possess either pluripotent stem cell or germ cell states and that three-dimensional aggregates of EBs initiate mES cell differentiation during prolonged culture in vitro. Therefore, we suggest that this EB system to some extent recapitulates the early developmental processes occurring in vivo.

Ratajczak, M. Z., E. K. Zuba-Surma, et al. (2008). "Hunt for pluripotent stem cell -- regenerative medicine search for almighty cell." J Autoimmun **30**(3): 151-62.

Regenerative medicine and tissue engineering are searching for a novel stem cell based therapeutic strategy that will allow for efficient treatment or even potential replacement of damaged organs. The pluripotent stem cell (PSC), which gives rise to cells from all three germ lineages, seems to be the most ideal candidate for such therapies. PSC could be extracted from developing embryos. However, since this source of stem cells for potential therapeutic purposes remains controversial, stem cell researchers look for PSC that could be isolated from the adult tissues or generated from already differentiated cells. True PSC should possess both potential for multilineage differentiation in vitro and, more importantly, also be able to complement in vivo blastocyst development. This review will summarize current approaches and limitations to isolate PSC from adult tissues or, alternatively, to generate it by nuclear reprogramming from already differentiated somatic cells.

Reddy, G. P., C. Y. Tiarks, et al. (1997). "Cell cycle analysis and synchronization of pluripotent hematopoietic progenitor stem cells." Blood **90**(6): 2293-9.

Hematopoietic stem cells purified from mouse bone marrow are quiescent with less than 2% of Lin- Hoechst(low)/Rhodamine(low) (Lin- Ho(low)/Rho(low)) and 10% to 15% of Lin-/Sca+ cells in S phase. These cells enter proliferative cycle and progress through G1 and into S phase in the presence of cytokines and 5% heat-inactivated fetal calf serum (HI-FCS). Cytokine-stimulated Lin- Ho(low)/Rho(low) cells took 36 to 40 hours to complete first division and only 12 hours to complete each of 5 subsequent divisions. These cells require 16 to 18 hours to transit through G0/G1 period and 28 to 30 hours to enter into mid-S phase during the first cycle. Up to 56% of Lin- Rho(low)/Ho(low) cells are high-proliferative potential (7 factor-responsive) colony-forming cells (HPP-CFC). At isolation, HPP-CFC are quiescent, but after 28 to 30 hours of culture, greater than 60% are in S phase. Isoleucine-deprivation of Lin- Ho(low)/Rho(low) cells in S phase of first cycle reversibly blocked them from entering into second cycle. After the release from isoleucine-block, these cells exhibited a G1 period of less than 2 hours and entered into mid-S phase by 12 hours. Thus, the duration of G1 phase of the cells in second cycle is 4 to 5 times shorter than that observed in their first cycle. Similar cell cycle kinetics are observed with Lin-/Sca+ population of bone marrow cells. Stem cell factor (SCF) alone, in the presence of HI-FCS, is as effective as a cocktail of 2 to 7 cytokines in inducing quiescent Lin-/Sca+ cells to enter into proliferative cycle. Aphidicolin treatment reversibly blocked cytokine-stimulated Lin-/Sca+ cells at G1/S boundary, allowing their tight synchrony as they progress through first S phase and enter into second G1. For these cells also, SCF alone is sufficient for their progression through S phase. These studies indicate a very short G1 phase for stem cells induced to proliferate and offer experimental approaches to synchronize murine hematopoietic stem cells.

Rietze, R. L., H. Valcanis, et al. (2001). "Purification of a pluripotent neural stem cell from the adult mouse brain." Nature **412**(6848): 736-9.

The adult mammalian central nervous system (CNS) contains a population of neural stem cells (NSCs) with properties said to include the generation of non-neural progeny. However, the precise identity, location and potential of the NSC in situ remain unclear. We purified NSCs from the adult mouse brain by flow cytometry, and directly examined the cells' properties. Here we show that one type of NSC, which expresses the protein nestin but only low levels of PNA-binding and HSA proteins, is found in both ependymal and subventricular zones and accounts for about 63% of the total NSC activity. Furthermore, the selective depletion of the population of this stem cell in querkopf mutant mice (which are deficient in production of olfactory neurons) suggests that it acts as a major functional stem cell in vivo. Most freshly isolated NSCs, when co-cultured with a muscle cell line, rapidly differentiated in vitro into myocytes that contain myosin heavy chain (MyHC). This demonstrates that a predominant, functional type of stem cell exists in the periventricular region of the adult brain with the intrinsic ability to generate neural and non-neural cells.

Sainteny, F., E. Larras-Regard, et al. (1990). "Thyroid hormones induce hemopoietic pluripotent stem cell differentiation toward erythropoiesis through the production of pluripoietin-like factors." Exp Cell Res **187**(1): 174-6.

We have previously reported that E pluripoietins are produced in mice after a single 20-mg injection of cytosine arabinoside (Ara-C) and that

they are able to initiate the determination of hemopoietic pluripotent stem cells (CFU-S) toward the erythrocytic lineage. However, the mechanism of E pluripoietin release is still unclear. Since the stimulating effect of thyroid hormone on erythropoiesis is well known, we postulated a link between this hormone and the E pluripoietins. In previous papers we demonstrated that L-triiodothyronine (LT3) exhibits the capacity of inducing CFU-S differentiation toward erythropoiesis in vitro. Two series of data presented here suggest that LT3 acts indirectly on CFU-S determination by promoting the release of E pluripoietin-like factors. First, the Ara-C injection which induces the production of E pluripoietins in mice also promotes an increase in the LT3 plasma level. Second, medium conditioned with bone marrow cells exposed in vitro for 90 min to LT3 (even though this medium does not contain LT3) has E pluripoietin-like effects, inducing CFU-S differentiation toward the erythrocytic lineage.

Salingcarnboriboon, R., H. Yoshitake, et al. (2003). "Establishment of tendon-derived cell lines exhibiting pluripotent mesenchymal stem cell-like property." Exp Cell Res **287**(2): 289-300.

Development of the musculoskeletal system requires coordinated formation of distinct types of tissues, including bone, cartilage, muscle, and tendon. Compared to muscle, cartilage, and bone, cellular and molecular bases of tendon development have not been well understood due to the lack of tendon cell lines. The purpose of this study was to establish and characterize tendon cell lines. Three clonal tendon cell lines (TT-E4, TT-G11, and TT-D6) were established using transgenic mice harboring a temperature-sensitive mutant of SV40 large T antigen. Proliferation of these cells was significantly enhanced by treatment with bFGF and TGF-beta but not BMP2. Tendon phenotype-related genes such as those encoding scleraxis, Six1, EphA4, COMP, and type I collagen were expressed in these tendon cell clones. In addition to tendon phenotype-related genes, expression of osteopontin and Cbfal was observed. These clonal cell lines formed hard fibrous connective tissue when implanted onto chorioallantoic membrane in ovo. Furthermore, these cells also formed tendon-like tissues when they were implanted into defects made in patella tendon in mice. As these tendon cell lines also produced fibrocartilaginous tissues in tendon defect implantation experiments, mesenchymal stem cell properties were examined. Interestingly, these cells expressed genes related to osteogenic, chondrogenic, and adipogenic lineages at low levels when examined by RT-PCR. TT-G11 and TT-E4 cells differentiated into either osteoblasts or adipocytes, respectively, when they were cultured in cognate differentiation medium. These observations indicated that the established tendon cell line possesses mesenchymal stem cell-like properties, suggesting the existence of mesenchymal stem cell in tendon tissue.

Scherer, C. A., J. Chen, et al. (1996). "Transcriptional specificity of the pluripotent embryonic stem cell." Cell Growth Differ **7**(10): 1393-401.

The specificity of gene expression in embryonic stem (ES) cells was analyzed both under in vitro culture conditions and during early embryogenesis. ES cells were infected with U3 beta geo, a U3 gene trap retrovirus that contains coding sequences for a beta-galactosidase-neomycin phosphotransferase hybrid protein. Integrated proviruses, which disrupted expressed cellular genes, were selected in the presence of G418. ES clones expressing regulated beta geo fusion genes were identified by changes in 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside staining after in vitro differentiation. Thirty-one of 191 clones tested (16%) exhibited regulated expression of beta geo protein. Seven genes disrupted by U3 beta geo were passed into the germline, and expression of the beta geo fusion genes was analyzed in vivo, including inserts disrupting the Eck and REX-1 genes. In each case, genes trapped in cultured ES cells were expressed in the inner cell mass of preimplantation embryos, and changes in lacZ expression during in vitro differentiation were also observed during early development. Thus, cultured ES cells maintain, to a considerable extent, the transcriptional specificity of the pluripotent cells of the preimplantation embryo. As a consequence, in vitro screens utilizing gene traps provide a rapid and accurate means to identify and disrupt developmentally regulated genes.

Schopperle, W. M. and W. C. DeWolf (2007). "The TRA-1-60 and TRA-1-81 human pluripotent stem cell markers are expressed on podocalyxin in embryonal carcinoma." Stem Cells **25**(3): 723-30.

We have previously identified the cell adhesion protein podocalyxin expressed in a human pluripotent stem cell, embryonal carcinoma (EC), which is a malignant germ cell. Podocalyxin is a heavily glycosylated membrane protein with amino acid sequence homology to the hematopoietic stem cell marker CD34. Since the initial discovery of podocalyxin in a cancerous stem cell, numerous new studies have identified podocalyxin in many different human cancers and in embryonic stem cells lines (ES) derived from human embryos. Embryonal carcinoma, as do all human pluripotent stem cells, expresses TRA-1-60 and TRA-1-81 antigens, and although their molecular identities are unknown, they are commonly used as markers of undifferentiated pluripotent human

stem cells. We report here that purified podocalyxin from embryonal carcinoma has binding activity with the TRA-1-60 and TRA-1-81 antibodies. Embryonal carcinoma cells treated with retinoic acid undergo differentiation and lose the TRA-1-60/TRA-1-81 markers from their plasma membrane surface. We show that podocalyxin is modified in the retinoic acid-treated cells and has an apparent molecular mass of 170 kDa on protein blots as compared with the apparent 200-kDa molecular weight form of podocalyxin expressed in untreated cells. Furthermore, the modified form of podocalyxin no longer reacts with the TRA-1-60/TRA-1-81 antibodies. Thus, embryonal carcinoma expresses two distinct forms of podocalyxin, and the larger version is a molecular carrier of the human stem cell-defining antigens TRA-1-60 and TRA-1-81.

Schwartz, P. H. (2008). "Training the next generation of pluripotent stem cell researchers." J Transl Med **6**: 40.

Human pluripotent stem cells (PSCs) have the unique properties of being able to proliferate indefinitely in their undifferentiated state and of being able to differentiate into any somatic cell type. These cells are thus posited to be extremely useful for furthering our understanding of both normal and abnormal human development, providing a human cell preparation that can be used to screen for new reagents or therapeutic agents, and generating large numbers of differentiated cells that can be used for transplantation purposes. PSCs in culture have a specific morphology and they express characteristic surface antigens and nuclear transcription factors; thus, PSC culture is very specific and requires a core skill set for successful propagation of these unique cells. Specialized PSC training courses have been extremely valuable in seeding the scientific community with researchers that possess this skill set.

Shi, Y. (2009). "Induced pluripotent stem cells, new tools for drug discovery and new hope for stem cell therapies." Curr Mol Pharmacol **2**(1): 15-8.

Somatic cell nuclear transfer or therapeutic cloning has provided great hope for stem cell-based therapies. However, therapeutic cloning has been experiencing both ethical and technical difficulties. Recent breakthrough studies using a combination of four factors to reprogram human somatic cells into pluripotent stem cells without using embryos or eggs have led to an important revolution in stem cell research. Comparative analysis of human induced pluripotent stem cells and human embryonic stem cells using assays for morphology, cell surface marker expression, gene expression profiling, epigenetic status, and differentiation potential have revealed a remarkable degree of similarity between these two pluripotent stem cell types. This mini-review summarizes these ground-breaking studies. These advances in reprogramming will enable the creation of patient-specific stem cell lines to study various disease mechanisms. The cellular models created will provide valuable tools for drug discovery. Furthermore, this reprogramming system provides great potential to design customized patient-specific stem cell therapies with economic feasibility.

Sommer, C. A., M. Stadtfeld, et al. (2009). "Induced pluripotent stem cell generation using a single lentiviral stem cell cassette." Stem Cells **27**(3): 543-9.

Induced pluripotent stem (iPS) cells can be generated using retroviral vectors expressing Oct4, Klf4, Sox2, and cMyc. Most prior studies have required multiple retroviral vectors for reprogramming, resulting in high numbers of genomic integrations in iPS cells and limiting their use for therapeutic applications. Here we describe the use of a single lentiviral vector expressing a "stem cell cassette" composed of the four transcription factors and a combination of 2A peptide and internal ribosome entry site technology, generating iPS cells from postnatal fibroblasts. iPS cells generated in this manner display embryonic stem cell-like morphology, express stem cell markers, and exhibit in vivo pluripotency, as evidenced by their ability to differentiate in teratoma assays and their robust contribution to mouse chimeras. Combining all factors into a single transcript achieves the most efficient reprogramming system to date and allows derivation of iPS cells with a single viral integration. The use of a single lentiviral vector for reprogramming represents a powerful laboratory tool and a significant step toward the application of iPS technology for clinical purposes.

Sperger, J. M., X. Chen, et al. (2003). "Gene expression patterns in human embryonic stem cells and human pluripotent germ cell tumors." Proc Natl Acad Sci U S A **100**(23): 13350-5.

Remarkably little is known about the transcriptional profiles of human embryonic stem (ES) cells or the molecular mechanisms that underlie their pluripotency. To identify commonalties among the transcriptional profiles of different human pluripotent cells and to search for clues into the genesis of human germ cell tumors, we compared the expression profiles of human ES cell lines, human germ cell tumor cell lines and tumor samples, somatic cell lines, and testicular tissue samples by using cDNA microarray analysis. Hierarchical cluster analysis of gene expression profiles showed that the five independent human ES cell lines clustered tightly together,

reflecting highly similar expression profiles. The gene expression patterns of human ES cell lines showed many similarities with the human embryonal carcinoma cell samples and more distantly with the seminoma samples. We identified 895 genes that were expressed at significantly greater levels in human ES and embryonal carcinoma cell lines than in control samples. These genes are candidates for involvement in the maintenance of a pluripotent, undifferentiated phenotype.

Stewart, M. H., M. Bosse, et al. (2006). "Clonal isolation of hESCs reveals heterogeneity within the pluripotent stem cell compartment." Nat Methods **3**(10): 807-15.

Human embryonic stem cell (hESC) lines are known to be morphologically and phenotypically heterogeneous. The functional nature and relationship of cells residing within hESC cultures, however, has not been evaluated because isolation of single hESCs is limited to drug or manual selection. Here we provide a quantitative method using flow cytometry to isolate and clonally expand hESCs based on undifferentiated markers, alone or in combination with a fluorescent reporter. This method allowed for isolation of stage-specific embryonic antigen-3-positive (SSEA-3+) and SSEA-3- cells from hESC cultures. Although both SSEA-3+ and SSEA-3- cells could initiate pluripotent hESC cultures, we show that they possess distinct cell-cycle properties, clonogenic capacity and expression of ESC transcription factors. Our study provides formal evidence for heterogeneity among self-renewing pluripotent hESCs, illustrating that this isolation technique will be instrumental in further dissecting the biology of hESC lines.

Tan, S. M., S. T. Wang, et al. (2007). "A UTF1-based selection system for stable homogeneously pluripotent human embryonic stem cell cultures." Nucleic Acids Res **35**(18): e118.

Undifferentiated transcription factor 1 (UTF1) was identified first in mouse embryonic stem cells and is also expressed in human embryonic and adult stem cells. UTF1 transcription ceases at the onset of differentiation, which clearly distinguishes it from less sensitive pluripotency markers, such as Oct4 or Nanog. We present here two transgenic hESC lines, named ZUN. Each line harbors one copy of the UTF1 promoter/enhancer driving a resistance gene and yielded highly homogeneous cultures under selection pressure, with a larger proportion of Oct4 and Sox2 positive cells. While ZUN cultures, like parental HUES8 cultures, retained the capacity to differentiate into tissues of all three germ layers using a SICD mouse teratoma model, they surprisingly exhibited an increased refractoriness to various differentiation cues in vitro. Together with its small size of only 2.4 kb for the entire cassette, these features render our selection system a powerful novel tool for many stem cell applications and human somatic cell reprogramming strategies.

Tanaka, T., S. Tohyama, et al. (2009). "In vitro pharmacologic testing using human induced pluripotent stem cell-derived cardiomyocytes." Biochem Biophys Res Commun **385**(4): 497-502.

The lethal ventricular arrhythmia Torsade de pointes (TdP) is the most common reason for the withdrawal or restricted use of many cardiovascular and non-cardiovascular drugs. The lack of an in vitro model to detect pro-arrhythmic effects on human heart cells hinders the development of new drugs. We hypothesized that recently established human induced pluripotent stem (hiPS) cells could be used in an in vitro drug screening model. In this study, hiPS cells were driven to differentiate into functional cardiomyocytes, which expressed cardiac markers including Nkx2.5, GATA4, and atrial natriuretic peptide. The hiPS-derived cardiomyocytes (hiPS-CMs) were analyzed using a multi electrode assay. The application of ion channel inhibitors resulted in dose-dependent changes to the field potential waveform, and these changes were identical to those induced in the native cardiomyocytes. This study shows that hiPS-CMs represent a promising in vitro model for cardiac electrophysiologic studies and drug screening.

Tarasov, K. V., G. Testa, et al. (2008). "Linkage of pluripotent stem cell-associated transcripts to regulatory gene networks." Cells Tissues Organs **188**(1-2): 31-45.

Knowledge of the transcriptional circuitry responsible for pluripotentiality and self-renewal in embryonic stem cells is tantamount to understanding early mammalian development and a prerequisite to determining their therapeutic potential. Various techniques have employed genomics to identify transcripts that were abundant in stem cells, in an attempt to define the molecular basis of 'stemness'. In this study, we have extended traditional genomic analyses to identify cis-elements that might be implicated in the control of embryonic stem cell-restricted gene promoters. The strategy relied on the generation of a problem-specific list from serial analysis of gene expression profiles and subsequent promoter analyses to identify frameworks of multiple cis-elements conserved in space and orientation among genes from the problem-specific list. Subsequent experimental data suggest that 2 novel transcription factors, B-Myb and Maz, predicted from these models, are implicated either in the maintenance

of the undifferentiated stem cell state or in early steps of differentiation.

Terstegge, S., B. H. Rath, et al. (2009). "Laser-assisted selection and passaging of human pluripotent stem cell colonies." J Biotechnol **143**(3): 224-30.

The derivation of somatic cell products from human embryonic stem cells (hESCs) requires a highly standardized production process with sufficient throughput. To date, the most common technique for hESC passaging is the manual dissection of colonies, which is a gentle, but laborious and time-consuming process and is consequently inappropriate for standardized maintenance of hESC. Here, we present a laser-based technique for the contact-free dissection and isolation of living hESCs (laser microdissection and pressure catapulting, LMPC). Following LMPC treatment, 80.6+/-8.7% of the cells remained viable as compared to 88.6+/-1.7% of manually dissected hESCs. Furthermore, there was no significant difference in the expression of pluripotency-associated markers when compared to the control. Flow cytometry revealed that 83.8+/-4.1% of hESCs isolated by LMPC expressed the surface marker Tra-1-60 (control: 83.9+/-3.6%). In vitro differentiation potential of LMPC treated hESCs as determined by embryoid body formation and multi-germlayer formation was not impaired. Moreover, we could not detect any overt karyotype alterations as a result of the LMPC process. Our data demonstrate the feasibility of standardized laser-based passaging of hESC cultures. This technology should facilitate both colony selection and maintenance culture of pluripotent stem cells.

Theilgaard-Monch, K., K. Raaschou-Jensen, et al. (2003). "Pluripotent and myeloid-committed CD34+ subsets in hematopoietic stem cell allografts." Bone Marrow Transplant **32**(12): 1125-33.

The present study compared the contents of pluripotent and lineage-committed hematopoietic progenitor cells (HPCs) in various types of allografts. Bone marrow (BM) allografts and single leukapheresis products (LPs) collected from G-CSF-mobilized donors contained similar amounts of pluripotent HPCs (CD34(+)CD38(-)) and total CD34(+) cells. However, the content of late-myeloid HPCs (CD34(+)CD33(+)CD15(+)) were significantly higher in BM grafts compared to LPs (P>0.02), whereas the contents of early-myeloid HPCs (CD34(+)CD33(+)CD15-) were 2.5-fold higher in LPs (P<0.03). In comparison to grafts from adult donors, cord blood (CB) grafts contained 26-65-fold lower amounts of early-myeloid HPCs (P<0.001), but only 8-12-fold lower contents of pluripotent HPCs (P<0.04). Additional findings demonstrated that among all tested parameters the numbers of early-myeloid HPCs were the most accurate measure of the total colony-forming cell (CFC) numbers in allografts. Hence, the earlier engraftment observed after transplantation of LPs compared to BM grafts might be explained by the higher content of early-myeloid HPCs/CFCs in LPs. Moreover, the slow engraftment following CB transplantation might not be affected essentially by the low number of myeloid HPCs, but rather by pluripotent HPCs. Finally, this study reports a new gating strategy for the enumeration of pluripotent CD34(+)CD38(-) subsets.

Vallier, L., T. Touboul, et al. (2009). "Signaling pathways controlling pluripotency and early cell fate decisions of human induced pluripotent stem cells." Stem Cells **27**(11): 2655-66.

Human pluripotent stem cells from embryonic origins and those generated from reprogrammed somatic cells share many characteristics, including indefinite proliferation and a sustained capacity to differentiate into a wide variety of cell types. However, it remains to be demonstrated whether both cell types rely on similar mechanisms to maintain their pluripotent status and to control their differentiation. Any differences in such mechanisms would suggest that reprogramming of fibroblasts to generate induced pluripotent stem cells (iPSCs) results in novel states of pluripotency. In that event, current methods for expanding and differentiating human embryonic stem cells (ESCs) might not be directly applicable to human iPSCs. However, we show here that human iPSCs rely on activin/nodal signaling to control Nanog expression and thereby maintain pluripotency, thus revealing their mechanistic similarity to human ESCs. We also show that growth factors necessary and sufficient for achieving specification of human ESCs into extraembryonic tissues, neuroectoderm, and mesendoderm also drive differentiation of human iPSCs into the same tissues. Importantly, these experiments were performed in fully chemically defined medium devoid of factors that could obscure analysis of developmental mechanisms or render the resulting tissues incompatible with future clinical applications. Together these data reveal that human iPSCs rely on mechanisms similar to human ESCs to maintain their pluripotency and to control their differentiation, showing that these pluripotent cell types are functionally equivalent.

Venable, A., M. Mitalipova, et al. (2005). "Lectin binding profiles of SSEA-4 enriched, pluripotent human embryonic stem cell surfaces." BMC Dev Biol **5**: 15.

BACKGROUND: Pluripotent human embryonic stem cells (hESCs) have the potential to

form every cell type in the body. These cells must be appropriately characterized prior to differentiation studies or when defining characteristics of the pluripotent state. Some developmentally regulated cell surface antigens identified by monoclonal antibodies in a variety of species and stem cell types have proven to be side chains of membrane glycolipids and glycoproteins. Therefore, to examine hESC surfaces for other potential pluripotent markers, we used a panel of 14 lectins, which were chosen based on their specificity for a variety of carbohydrates and carbohydrate linkages, along with stage specific embryonic antigen-4 (SSEA-4), to determine binding quantitation by flow cytometry and binding localization in adherent colonies by immunocytochemistry. RESULTS: Enriching cells for SSEA-4 expression increased the percentage of SSEA-4 positive cells to 98-99%. Using enriched high SSEA-4-expressing hESCs, we then analyzed the binding percentages of selected lectins and found a large variation in binding percentages ranging from 4% to 99% binding. Lycopersicon (tomato)esculetum lectin (TL), Ricinus communis agglutinin (RCA), and Concanavalin A (Con A) bound to SSEA-4 positive regions of hESCs and with similar binding percentages as SSEA-4. In contrast, we found Dolichos biflorus agglutinin (DBA) and Lotus tetragonolobus lectin (LTL) did not bind to hESCs while Phaseolus vulgaris leuco-agglutinin (PHA-L), Vicia villosa agglutinin (VVA), Ulex europaeus agglutinin (UEA), Phaseolus vulgaris erythro-agglutinin (PHA-E), and Maackia amurensis agglutinin (MAA) bound partially to hESCs. These binding percentages correlated well with immunocytochemistry results. CONCLUSION: Our results provide information about types of carbohydrates and carbohydrate linkages found on pluripotent hESC surfaces. We propose that TL, RCA and Con A may be used as markers that are associated with the pluripotent state of hESCs because binding percentages and binding localization of these lectins are similar to those of SSEA-4. Non-binding lectins, DBA and LTL, may identify differentiated cell types; however, we did not find these lectins to bind to pluripotent SSEA-4 positive hESCs. This work represents a fundamental base to systematically classify pluripotent hESCs, and in future studies these lectins may be used to distinguish differentiated hESC types based on glycan presentation that accompanies differentiation.

Walsh, J. and P. W. Andrews (2003). "Expression of Wnt and Notch pathway genes in a pluripotent human embryonal carcinoma cell line and embryonic stem cell." Apmis **111**(1): 197-210; discussion 210-1.

Embryonal carcinoma (EC) cells, the pluripotent stem cells of teratocarcinomas, show many similar-ities to embryonic stem (ES) cells. Since EC cells are malignant but their terminally differentiated derivatives are not, understanding the molecular mechanisms that regulate their differentiation maybe of value for diagnostic and therapeutic purposes. We have examined the expression of multiple components of two developmentally important cell-cell signalling pathways, Wnt and Notch, in the pluripotent human EC cell line, NTERA2, and the human ES cell line, H7. Both pathways have well-documented roles in controlling neurogenesis, a process that occurs largely in response to retinoicacid (RA) treatment of NTERA2 cultures and spontaneously in H7 cultures. In NTERA2, many ofthe genes tested showed altered transcriptional regulation following treatment with RA. These include members of the frizzled gene family (FZDI, FZD3, FZD4, FZD5, FZD6), encoding receptors forWnt proteins, the Frizzled Related Protein family (SFRPI, SFRP2, FRZB, SFRP4), encoding solubleWnt antagonists and also ligands and receptors of the Notch pathway (Dlkl, Jaggedl; Notchl, Notch2, Notch3). Few differences were found in the repertoire of Wnt and Notch pathway genes expressed by NTERA2 EC cells and H7 ES cells. We present a model in which interactions between and regulation of Wnt and Notch signalling are important in maintaining EC/ES stem cells and also controlling their differentiation.

Werbowetski-Ogilvie, T. E. and M. Bhatia (2008). "Pluripotent human stem cell lines: what we can learn about cancer initiation." Trends Mol Med **14**(8): 323-32.

Although the cancer stem cell (CSC) hypothesis has become an attractive model to account for tumor recurrence, failure to define a cell of origin has created the need to explore alternative models for cancer initiation and maintenance. Recent studies have linked an embryonic stem cell (ESC)-like gene signature with poorly defined high-grade tumors. Here, we review advances in the ESC field with an emphasis on how human pluripotent stem cells (hPSCs) can be used to define early tumorigenic events, including potential miRNA and epigenetic targets, as well as proto-oncogene and tumor suppressor networks that might facilitate hierarchal transformation. These studies allow for investigation of cancer initiation in a manner that cannot be achieved using primary tumors, where only retrospective evaluation of CSC development is possible. By comparing transformed hPSCs with their normal counterparts, we hope to develop novel cell-specific therapies that selectively target CSCs.

Wierenga, P. K. and A. W. Konings (1990). "Effect of a hyperthermic treatment on the pluripotent haemopoietic stem cell in normal and anaemic mice." Int J Hyperthermia **6**(4): 793-800.

Up to now, the hyperthermic sensitivity of pluripotent haemopoietic stem cells is unknown, and the few existing data from reports in the literature are conflicting. There are two main drawbacks in the set-up of those studies: (1) only CFU-S day 9 results were presented, whereas it is questionable if this assay gives a true reflection of the pluripotent stem cell, and (2) no attention has been paid to heat effects on the seeding efficiency, i.e. the amount of stem cells which will lodge in the spleen. The present study focused on the procedural differences and compared the results of a hyperthermic treatment (60 min, 42 degrees C) on the stem cells, assayed with the CFU-S day 9 and the CFU-S day 12 method, using the following three stem cell suspensions, all differing in their proliferative activity: bone marrow from normal mice and bone marrow and spleen cells from anaemic mice. Furthermore, we investigated the seeding efficiency before and after heat treatment. Resting stem cells, assayed with the CFU-S day 12 method, turned out to be resistant to hyperthermia as compared with the active cycling stem cells, while with the CFU-S day 9 assay the stem showed the same thermosensitivity in the two bone marrow suspensions. The active cycling stem cells do not significantly differ in thermosensitivity, in CFU-S day 9 and day 12 assays, although there is a difference between bone marrow and spleen. Hyperthermia appears to influence the seeding efficiency for spleen CFU-S; an increase of 1.73 was observed.(ABSTRACT TRUNCATED AT 250 WORDS)

Wobus, A. M., H. Holzhausen, et al. (1984). "Characterization of a pluripotent stem cell line derived from a mouse embryo." Exp Cell Res **152**(1): 212-9.

A pluripotent, karyotypically normal, male culture line ESC-BLC 1 of embryonal stem cells was established from delayed mouse blastocysts of strain 129/ter Sv. The cell line was isolated after cultivation of inner cell mass cells on X-irradiated feeder layer of mouse embryonal fibroblasts. The pluripotent status of the cell line was confirmed by in vivo and in vitro differentiation. For in vivo differentiation, cells were injected subcutaneously into syngeneic mice. The resulting tumors contained various tissues, derivatives of all three primary germ layers. In vitro cultivated pluripotent stem cells differentiated into endoderm-like, neuronal-like and tubular structures. Determination of alkaline phosphatase in cell line ESC-BLC 1 yielded a high specific activity; G-banding of metaphases revealed a normal, male karyotype.

Wobus, A. M., U. Kiessling, et al. (1984). "DNA transformation of a pluripotent mouse embryonal stem cell line with a dominant selective marker." Cell Differ **15**(2-4): 93-7.

The mouse embryonal stem cell line BLC 1 growing on feeder layer was treated with a calcium phosphate/DNA precipitate prepared with DNA of plasmid pAG60 which harbors the Tn5-derived neo gene, thus encoding resistance to G418, an aminoglycoside antibiotic. Transfection performed on feeder layer resulted in the formation of G418-resistant clones T1 and T2/K26. The stable integration of the transformed neo gene was confirmed by dot hybridization in all descendant cultures of clones T1 and T2/K26 as well as in the tumors derived from them. In vivo and in vitro differentiation revealed the pluripotent status of the transformants. Tumors derived from T1 and T2/K26 contained various tissues with derivatives of all three primary germ layers.

Yuasa, S. and K. Fukuda (2008). "Recent advances in cardiovascular regenerative medicine: the induced pluripotent stem cell era." Expert Rev Cardiovasc Ther **6**(6): 803-10.

Induced pluripotent stem (iPS) cells have recently been established by transfecting mouse and human fibroblasts with the transcription factors Oct3/4, Sox2, Klf4 and c-Myc, known to be expressed at high levels in embryonic stem (ES) cells. These cells have great potential in regenerative medicine as they have the capacity to differentiate into all three germ layer-derived cells and are syngeneic. The differentiation of ES cells into cardiomyocytes mimics the early processes involved in heart development. Recent studies describe the contribution of various growth factors and corresponding inhibitors to heart development during embryogenesis. Bone morphogenetic proteins, Wnt protein and Notch signals play critical roles in heart development in a context- and time-dependent manner. Consistent with ES cells, the exposure of iPS cells to such growth factors is hypothesized to augment differentiation into cardiomyocytes. The combination of iPS cells and appropriate developmental signal information has the potential for providing the foundations for future regenerative medicine.

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9/10/2011