STEM CELLS

Definition?
Where from?
What's for?
Where are we?

1

Definition?

It is now well accepted that a stem cell must fulfill three criteria:

1. First, it must be capable of self-renewal, i.e., undergoing symmetric or asymmetric divisions through which the stem cell population is maintained.
2. A single cell must be capable of multilineage differentiation.
3. In vivo functional reconstitution of a given tissue.

The definition of 'stem cell' is essentially **functional**: ''rather than referring to a discrete cellular entity, a stem cell most accurately refers to a biological function that can be induced in many distinct types of cells, even differentiated cells''

2

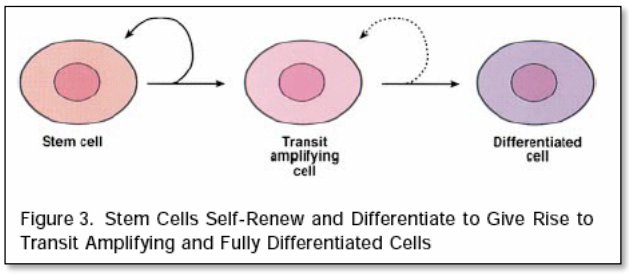


Figure 3. Stem Cells Self-Renew and Differentiate to Give Rise to Transit Amplifying and Fully Differentiated Cells

✓stem cells are **relatively uncommon**, with frequencies varying from roughly 0.0001% to roughly 5% of the total cells in a tissue—accordingly, tissue-specific stem cells may be difficult to isolate;

✓stem **cells cycle relatively slowly**, and often we see transit amplifying (TA) cells dividing more often than stem cells;

✓stem cell activity is governed by the cells' microenvironment or '**niche**', comprising cell-adhesion molecules, cell–cell signals and growth factors; and

✓more controversially, stem cell populations are **selfmaintaining,** in that each stem cell division, on average, generates one stem cell and one TA cell, or each two stem cell divisions, on average, generate two stem cells and two TA cells 3

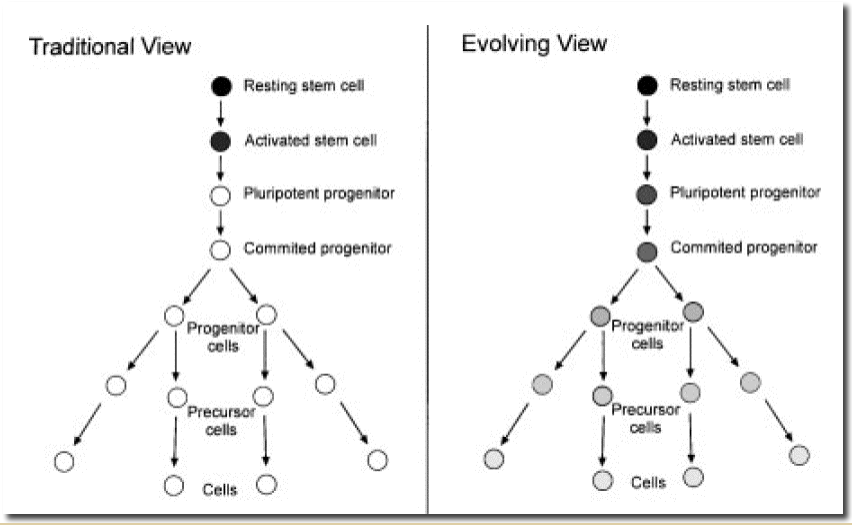
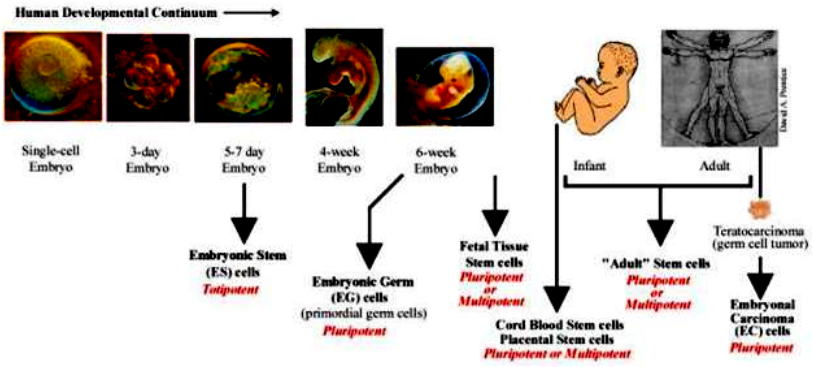


Figure 1. Two concepts of stem cells. On the left is the traditional view of stem cells, showing an irreversible loss of potency in maturing stem cells. On the right is an evolving view postulated by Blau et al. whereby stemness is a biological function that progressively degenerates over time but remains potentially recruitable within even differentiated cells in particular contexts. Redrawn with substantial modification from Fig. 7 in Blau HM, Brazelton TR, Weimann JM. 2001. The evolving concept of a stem cell: Entity or function? Cell 105:829–841, with permission of Elsevier.

STEM CELLS

Where from?



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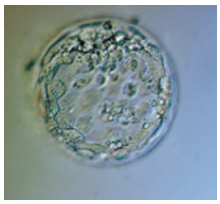
A human embryo, at the “blastocyst” stage, used to create new stem cell lines.



www.sciencemag.org SCIENCE VOL 282 6 NOVEMBER 1998

Embryonic Stem Cell Lines Derived from Human Blastocysts

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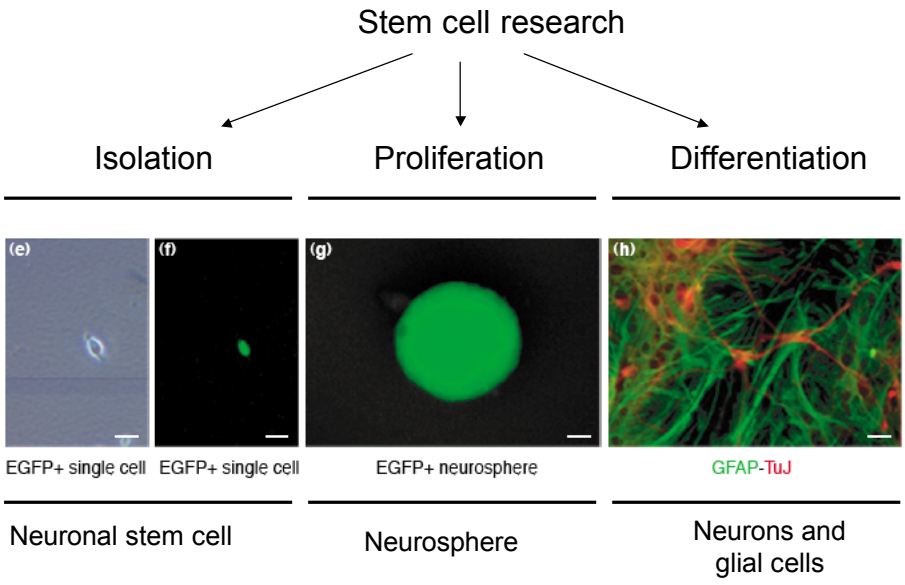

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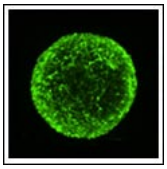
TRENDS in Neurosciences Vol.26 No.7 July 2003

Neural Stem Cells - The ins and outs of Neurospheres

Today, the neurosphere assay is the most common way of isolating and expanding neural stem cells in vitro. So what are "neurospheres"? Neural stem cells can be isolated by taking brain tissue from, for example, embryonic day 14.5 cortex, followed by mechanical dissociation and then plating on culture dishes containing serum-free defined media with EGF. After about 6 days free floating colonies of nestin positive cells can be seen, with each colony containing approximately 200-500 cells (Figure 1). They can be expanded either by cutting the neurospheres into 4-8 pieces or by dissociation into single cells followed by reculturing. If neurospheres are plated on laminin, the EGF withdrawn and



Serum added, they can be seen to differentiate into the three main cell types of the brain, astrocytes, oligodendrocytes and neurons. In particular cells at the periphery of the neurosphere begin to migrate and differentiate while cells at the center remain undifferentiated. By selecting the plating matrix and growth factors the proportion of the three cell types can be controlled (1). For example, NB3 has recently been shown the promote oligodendrocyte formation from mouse brain neurosperes (2).

(1) Nature Biotechnology19, (2001) 475-479. *(2) J. Biol. Chem: 279, (2004) 25858-25865.*

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Neurospheres are formed because daughter neural stem cells remain attached to their mothers through many rounds of cell division. So are all the cells in a neurosphere the same? This is an important question that has only been partially answered. Neurospheres are comprised of a heterogenous mix of neural stem cells, neural progenitors, differentiated cells and extracellular matrix proteins (7). Clonal analysis where single cells are isolated and characterised has to be carried out to investigate the exact make-up of neurospheres. Culturing neurospheres from single isolated cells is one way of doing clonal analysis, however, growing cells at very low density may lead to selection of unique populations that are not representative. We will have to identify novel ways of following neural stem cell lineages. Nevertheless, it is clear that the neurosphere assay is a very powerful way to propagate neural stem cells and model neurodevelopment.

Looking to the future, it will be very important to understand the relationship between the cells of the colony and exactly how many different cell types there are in any one particular neurosphere. The composition of neurospheres isolated from different regions of the brain or under different growth conditions will also be important to establish.

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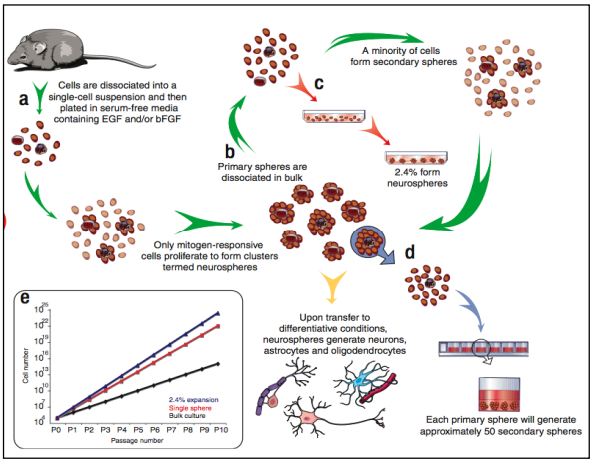
Figure 1. Detection and expansion of stem and progenitor cells using the neurosphere assay.

(a) When embryonic or adult primary CNS tissue is transferred to neurosphere-generating conditions, both neural stem cells and non-stem precursor cells survive.

(b) Following dissociation and replating, primary neurosphere–derived neural stem cells and non-stem precursor cells proliferate to form secondary spheres. (c) When plated at a clonal density, approximately 2.4% of cells generate a neurosphere. (d) When individual clonally derived neurospheres are mechanically dissociated into a single-cell suspension and plated into a single well (at one sphere per well), each sphere will generate about 50 secondary spheres. (e) Serial passage growth curves based on an actual bulk culture (black line), 50-fold expansion as determined by single-sphere dissociation data (red line) and a theoretical stem cell frequency of 2.4% (blue line).



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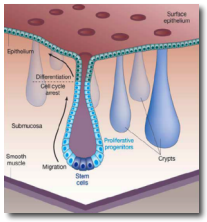
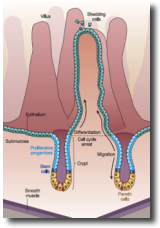
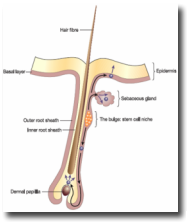
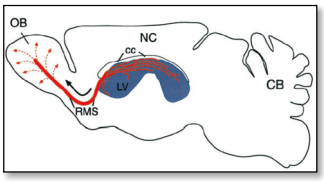
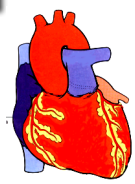
The most extensively studied adult stem cell is the hematopoietic stem cell (HSC). Neural stem cells (NSC) give rise to neurons, astrocytes, and oligodendrocytes. Mesenchymal stem cells (MSC) differentiate into fibroblasts, osteoblasts, chondroblasts, adipocytes, and skeletal muscle. Other stem cells have been identified, including gastrointestinal stem cells, epidermal stem cells, and hepatic stem cells (also called oval cells).

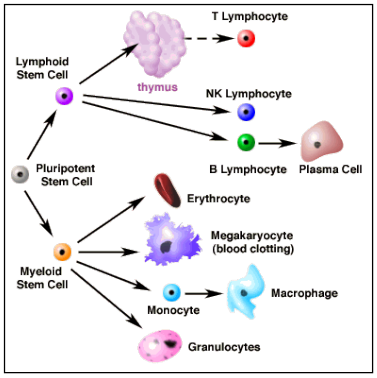




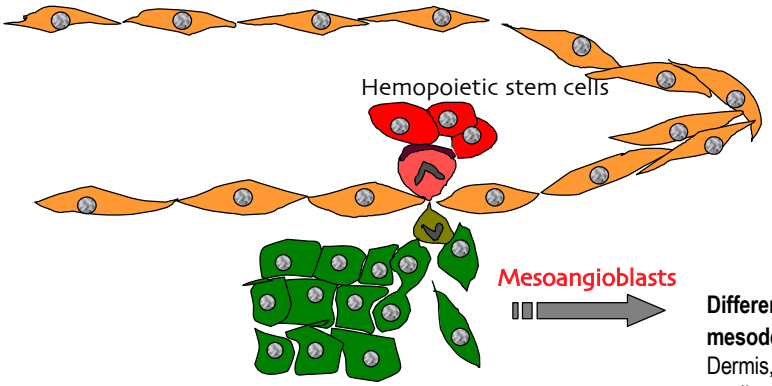








Vessel associated progenitor cells may enter surrounding tissues and adopt the local fate



Differentiate to most mesodermal cell types: Dermis, tendons, Skeletal, cardiac and smooth muscle, bone, bone marrow, cartilage...

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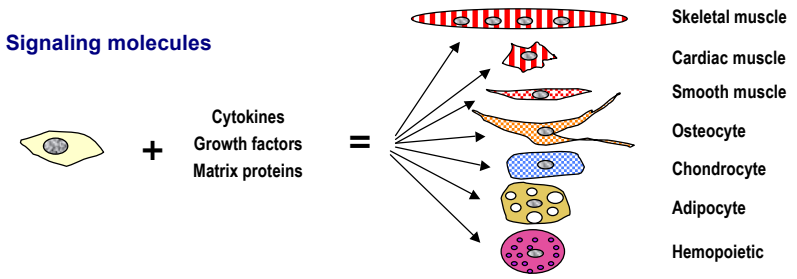
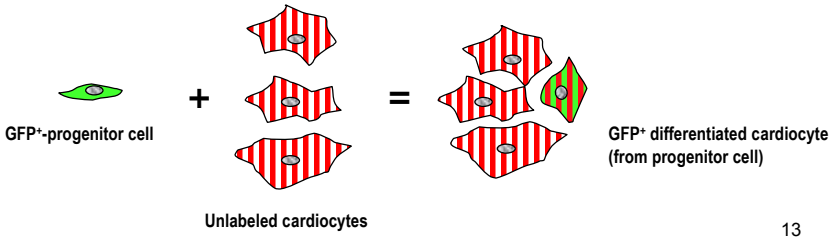
Different signaling processes may activate different fates in pluripotent progenitors



Co-culture test for pluripotency: Progenitor cells are recruited to a given differentiation pathway, e.g. cardiogenesis, by neighboring differentiating cells (e.g. cardiocytes)



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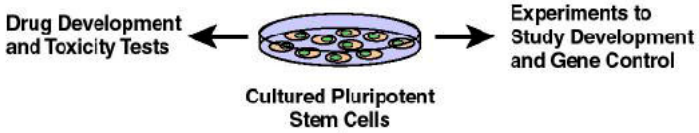
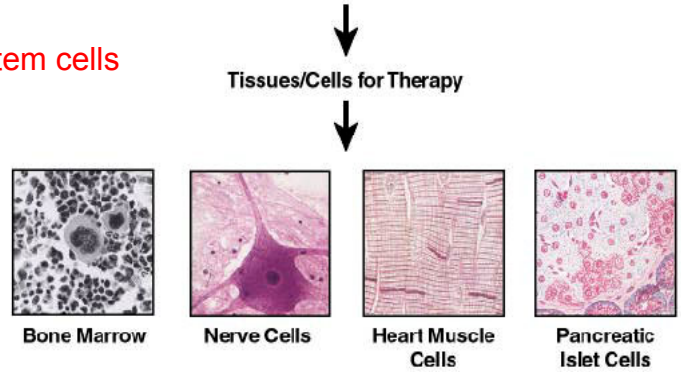
What's for?

The promise of Stem Cell Research

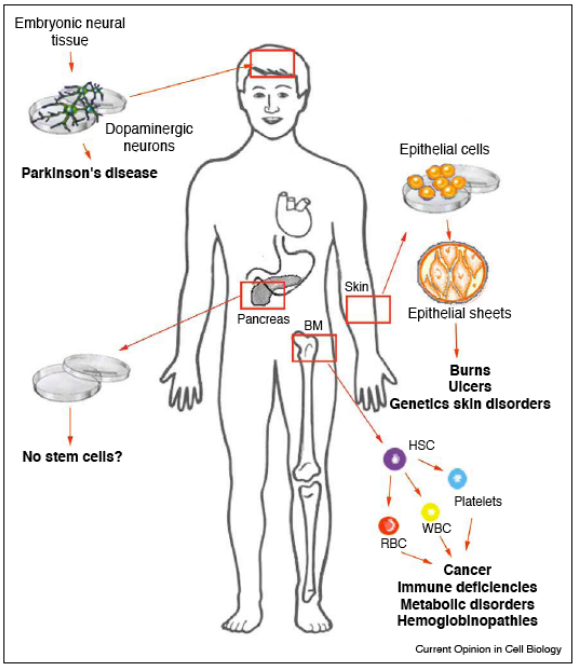
Embryonic stem cells



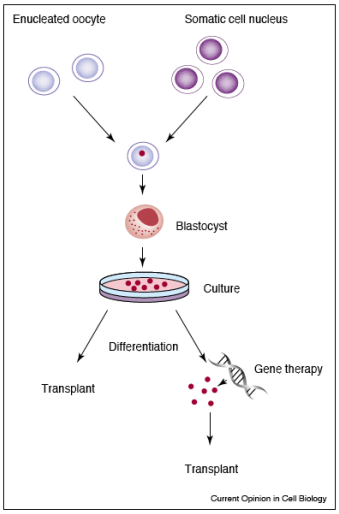
Adult stem cells



Reconstructive therapy



Gene therapy



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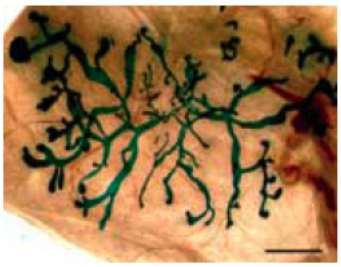
Nature January 2006

Generation of a functional mammary gland from a single stem cell

Mark Shackleton[1,2], François Vaillant[1,2], Kaylene J. Simpson[3]†, John Stingl[4,5], Gordon K. Smyth[1], Marie-Liesse Asselin-Labat[1,2], Li Wu[1], Geoffrey J. Lindeman[1,2] & Jane E. Visvader[1,2]

A LacZ- outgrowth arising from transplantation of 13 visualized, double-sorted Lin-CD29^{hi}CD24+ cells.



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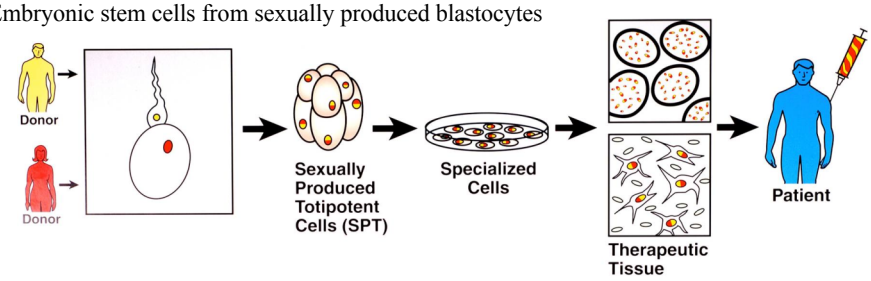
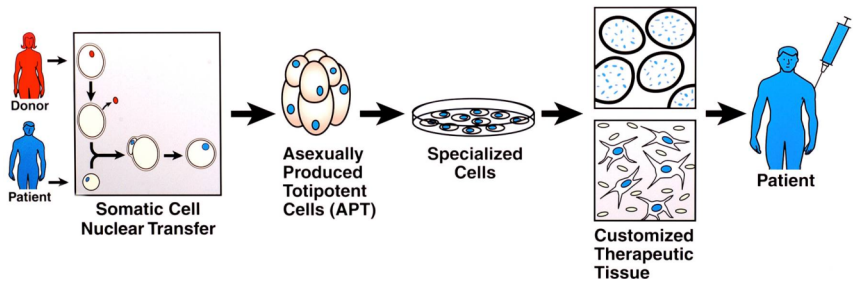
Embryonic stem cells from sexually produced blastocytes



Embryonic stem cells from asexually produced blastocyte (somatic cell nuclear transfer)



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Process of generating embryonic stem cells

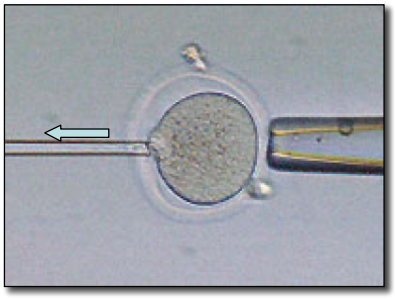
The first step in the process of generating embryonic stem cells is to remove the nucleus from an unfertilized egg cell

(A). We use a suction pipette (B) to hold the egg cell steady and a glass needle (C) to remove the cellís nucleus.

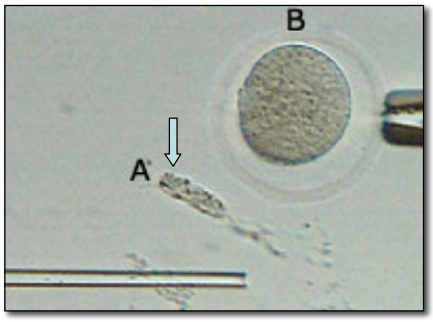
Because the egg cell is only 100 micrometers, or one-tenth of a millimeter, wide, we monitor this fine surgical extraction with a microscope (see previous image).

18

Remove the nucleus from an unfertilized egg cell



We have gently pushed the glass needle through the tough shell that surrounds the egg cell. In nature, the zona pellucida, as this shell is known, protects the egg as it travels down the fallopian tube on its way to the uterus; it also regulates fertilization so that only a single sperm may enter the egg. Here, the glass needle is in the process of removing the nucleus from within the egg. If you look closely at the tip of the needle, you can just make out the genetic material being drawn out.



We have released the egg cell's nucleus (A) outside of the egg. This nuclear material will no longer be needed. What remains is an "enucleated" egg (B) that still contains protein, RNA molecules, and other important factors that will ultimately help to establish embryonicstem cells.

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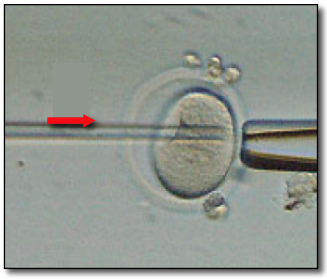
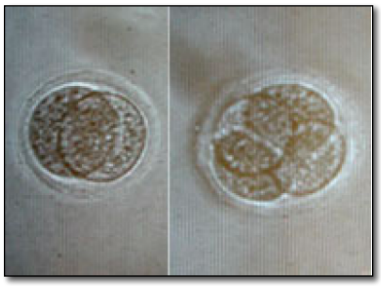
Inject the nucleus (at arrow) from a donor cell into the enucleated egg cell



In the future, such a donor cell might be a skin cell from a disease sufferer whom doctors hope to treat using the patient's own stem cells grown in culture; the procedure would be essentially the same as we're showing here. Once again we ease the tip of the glass needle through the zona pellucida and deep into the enucleated egg cell, where we then deposit the donor nucleus.

”Activate" the unfertilized egg cell using a chemical or electrical treatment that stimulates cellular division.



The first division results in two cells (left image), the next makes four cells, and so on. This structure is now termed an embryo.

20

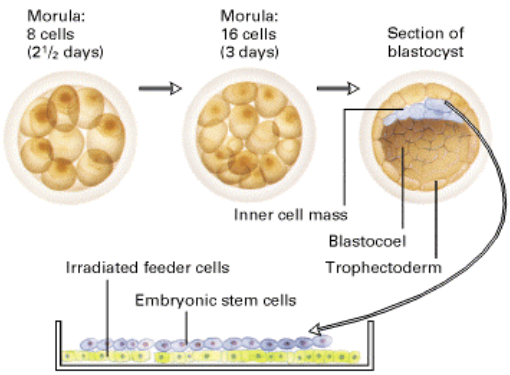
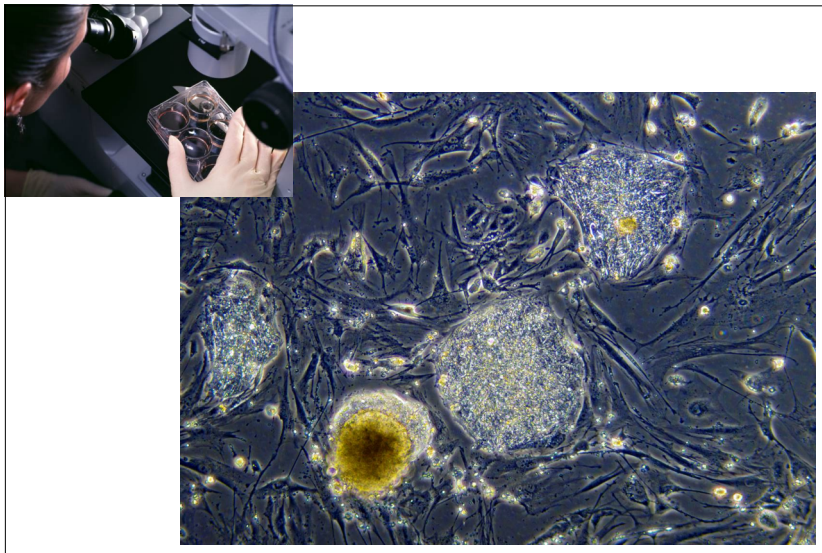


Figure 8-32. Preparation of embryonic stem (ES) cells. Fertilized mouse eggs divide slowly at first; after 41/2 days, they form the blastocyst, a hollow structure composed of about 100 cells surrounding an inner cavity called the blastocoel. Only ES cells, which constitute the inner cell mass, actually form the embryo. Other cells form the trophectoderm, which gives rise to the membranes (amnion and placenta) by which the embryo is attached to the uterine wall. Embryonic stem cells can be removed from the blastocyst and grown on lethally irradiated "feeder cells." [See E. Robertson et al., 1986, Nature 323:445.]

21



Microscopic 10x view of a colony of undifferentiated human embryonic stems cells being studied in developmental biologist James Thomson's research lab. The embryonic stem cell colonies are the rounded, dense masses of cells. The flat, elongated cells in between the embryonic stem cell colonies are fibroblasts that are used as a "feeder layer" on which the embryonic stem cells are grown. (Source: University of Wisconsin-Madison.)

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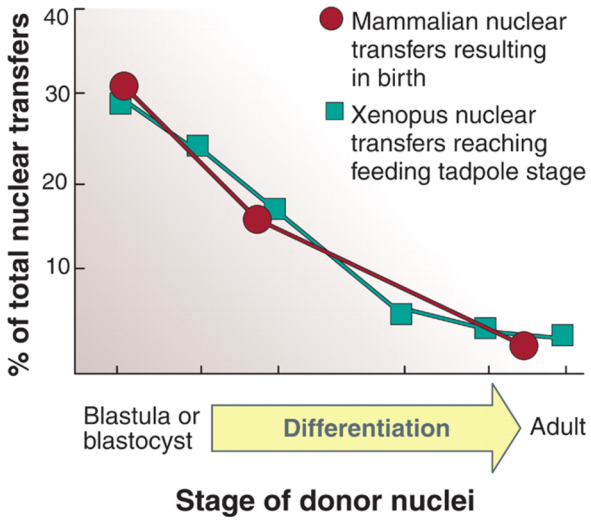
Fig. 2. Nuclear transfer success decreases as donor cells differentiate (3, 8)



J. B. Gurdon et al., Science 322, 1811 -1815 (2008)

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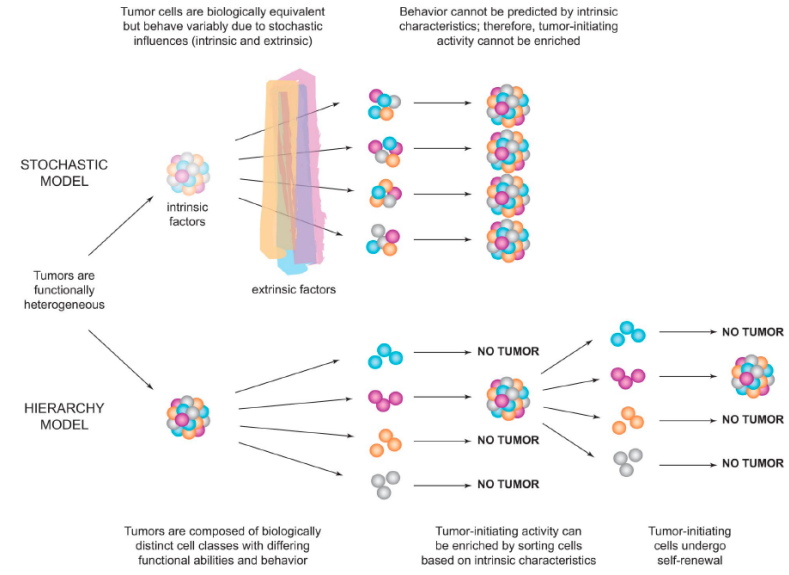

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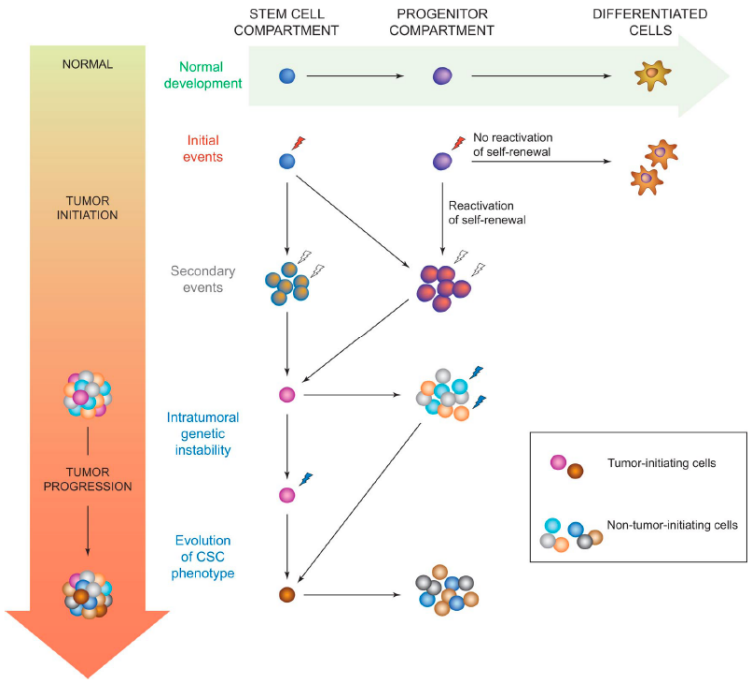

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Leukaemia stem cells and the evolution of cancer-stem-cell research

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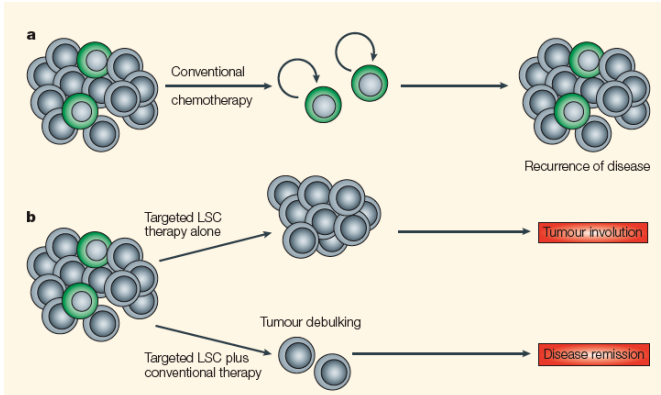

Figure 4 | **Targeting leukaemia stem cells. a** | At present, treatment for leukaemia uses chemotherapeutic agents that target all leukaemia cells (grey), based on properties such as their increased proliferation and entry into the cell cycle. However, it is likely that this approach spares the population of leukaemia stem cell (LSCs; green), which are responsible for the continued growth and propagation of the tumour. In many instances, this leads to recurrence of the disease. **b** | A greater understanding of LSC biology will allow us to design therapeutic agents that specifically target the LSC populations. Such therapies used alone, or in combination with conventional chemotherapeutic agents that reduce tumour burden, should lead to tumour involution or disease remission, respectively. Both of these approaches could improve both initial response rates and overall survival, through a decrease in the relapse of disease.

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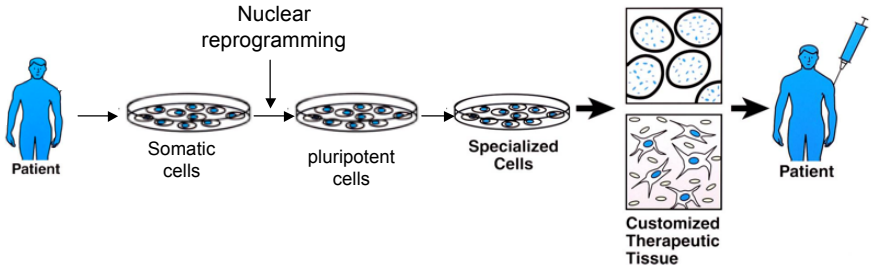
Nuclear Reprogramming in cells



27

Normal process of cell differentiation during development from a fertilized egg to adult cells or tissues.

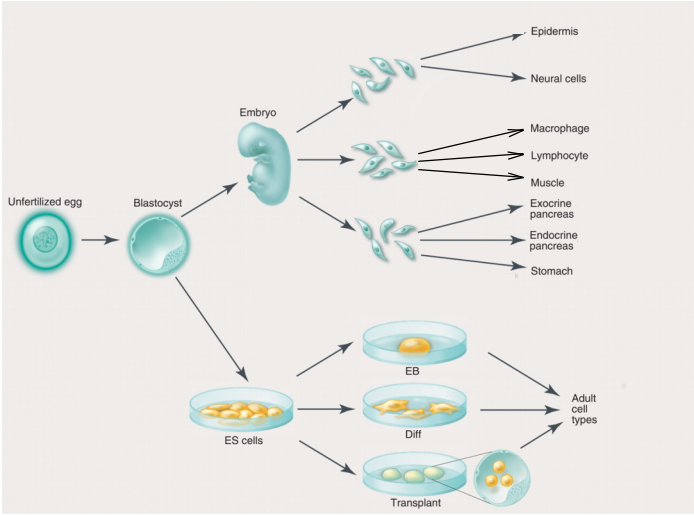


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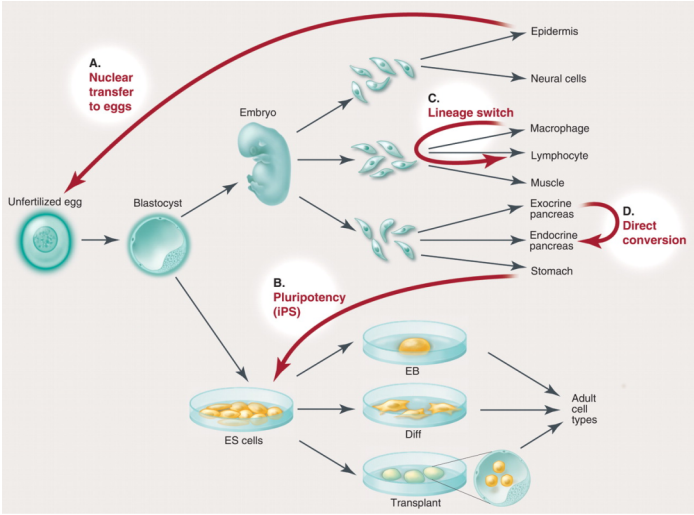
Fig. 5. Four experimental routes for nuclear reprogramming



(A) by nuclear transfer to eggs

(B) by induced pluripotency iPS

(C) by lineage switching back to a branch point and out again in a different direction

(D) by direct conversion

The lower part of the figure shows reprogramming by the generation of ES cells; these can be aggregated into an embryoid body (EB), made to differentiate in culture (diff), or transplanted to a blastocyst. In each case, various types of adult cells can be formed.

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Science AAAS

Cell *126*, 663–676, August 25, 2006

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

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

"We hypothesized that the factors that play important roles in the maintenance of ES cell identity also play pivotal roles in the induction of pluripotency in somatic cells".

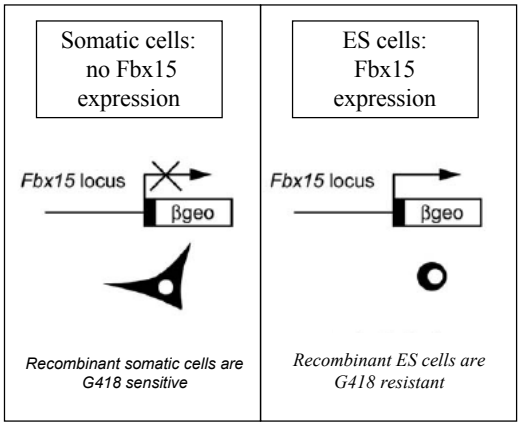
30

We selected 24 genes as candidates for factors that induce pluripotency in somatic cells, based on our hypothesis that such factors also play pivotal roles in the maintenance of ES cell identity. For b-catenin, c-Myc, and Stat3, we used active forms,
S33Y-b-catenin (Sadot et al., 2002), T58A-c-Myc (Chang et al., 2000), and Stat3-C (Bromberg et al., 1999), respectively.
Because of the reported negative effect of Grb2 on pluripotency (Burdon et al., 1999; Cheng et al., 1998), we included its dominant-negative mutant Grb2DSH2 (Miyamoto et al., 2004) as 1 of the 24 candidates.

31

Assay system in which the induction of the pluripotent state could be detected as resistance to G418.
We inserted a βgeo cassette (a fusion of the β -galactosidase and neomycin resistance genes) into the mouse Fbx15 gene by homologous recombination (Tokuzawa et al., 2003). Although specifically expressed in mouse ES cells and early embryos, Fbx15 is dispensable for the maintenance of pluripotency and mouse development

ES cells homozygous for the β geo knockin construct (Fbx15 β geo/ β geo) were resistant to extremely high concentrations of G418 (up to 12 mg/ml), whereas **somatic cells** derived from Fbx15bgeo/bgeo mice were sensitive to a normal concentration of G418 (0.3 mg/ml). We expected that even partial activation of the Fbx15 locus would result in resistance to normal concentrations of G418.



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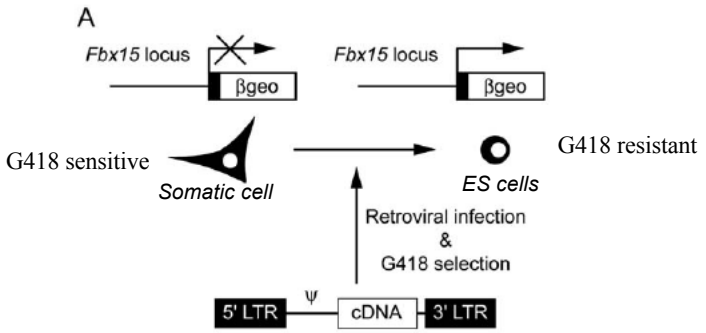

Retroviral infection with constructs for each of those factors known to be important for plutipotency

Ecat1	Klf4
Dppa5	β-catenin
Fbxo15	c-Myc
Nanog	Stat3
ERas	Grb2
Dnmt3l	Ecat8
Oct3/4	Gdf3
Sox2	Sox15
Rex1	Dppa4
Utf1	Dppa2
Tcl1	Fthl17
Dppa3	Sall4

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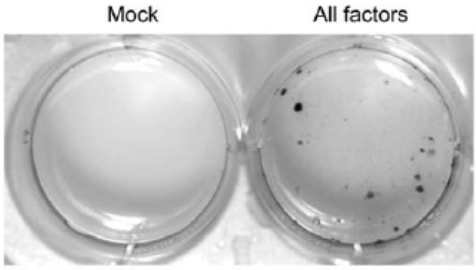
We introduced each of the 24 candidate genes into mouse embryonic fibroblasts (MEFs) from Fbx15 b geo/ b geo embryos by retroviral transduction. Transduced cells were then cultured on STO feeder cells in ES cell medium containing G418 (0.3 mg/ml).



G418-resistant colonies were observed 16 days after transduction with a **combination of 24 factors**. Cells were stained with crystal violet.

no drug-resistant colonies with any single factor, indicating that no single candidate gene was sufficient to activate the Fbx15 locus

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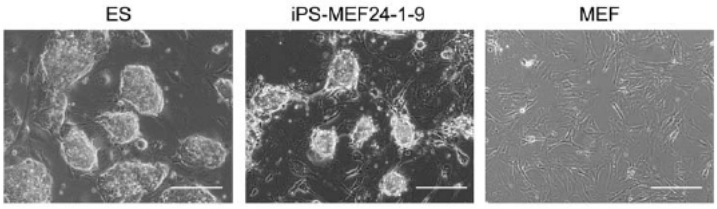
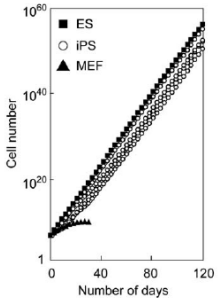
Of the 12 clones for which we continued cultivating under selection, 5 clones exhibited morphology similar to ES cells, including a round shape, large nucleoli, and scant cytoplasm



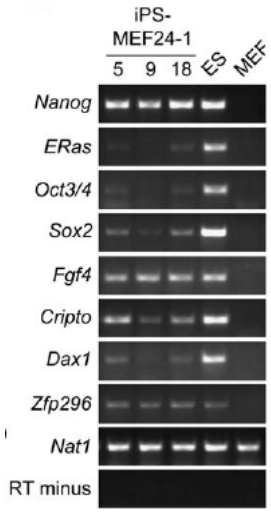
iPS-MEF24 = "pluripotent stem cells induced from MEFs by 24 factors

4 of these clones possessed ES cell-like morphology and proliferation properties



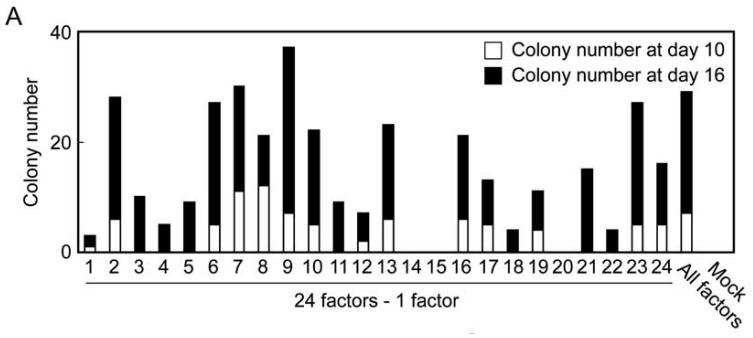
Reverse transcription PCR (RT-PCR) analysis revealed that the iPS-MEF24 clones expressed ES cell markers, including Oct3/4, Nanog, E-Ras, Cripto, Dax1, and Zfp296 and Fgf4

These data indicate that some combination of these 24 candidate factors induced the expression of ES cell marker genes in MEF culture.



36

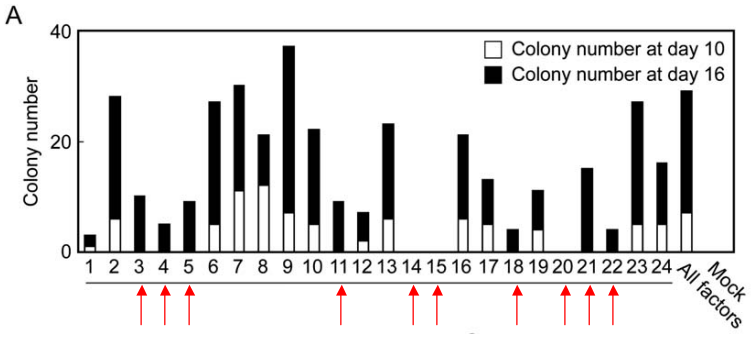
we examined the effect of withdrawal of individual factors from the pool of transduced candidate genes on the formation of G418-resistant colonies



Effect of the removal of individual factors from the pool of 24 transduced factors on the formation of G418-resistant colonies. Fbx15bgeo/bgeo MEFs were transduced with the indicated factors and selected with G418 for 10 days (white columns) or 16 days (black columns).

37

we examined the effect of withdrawal of individual factors from the pool of transduced candidate genes on the formation of G418-resistant colonies



Effect of the removal of individual factors from the pool of 24 transduced factors on the formation of G418-resistant colonies. Fbx15bgeo/bgeo MEFs were transduced with the indicated factors and selected with G418 for 10 days (white columns) or 16 days (black columns).

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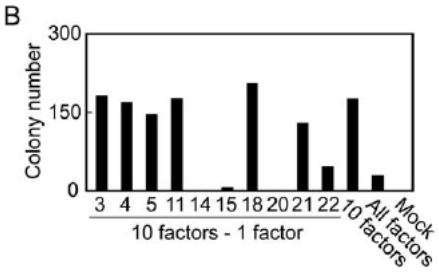
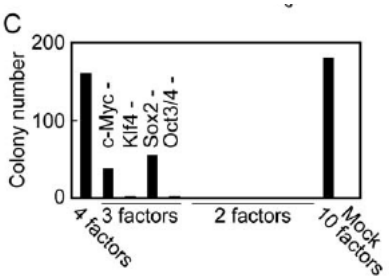


Effect of the removal of individual factors from the selected 10 factors on the formation of G418-resistant colonies 16 days after transduction

Effect of the transduction of pools of four, three, and two factors on the formation of G418-resistant colonies 16 days after transduction



These data demonstrate that iPS cells can be induced from MEF culture by the introduction of four transcription factors, Oct3/4, Sox2, c-Myc, and Klf4.

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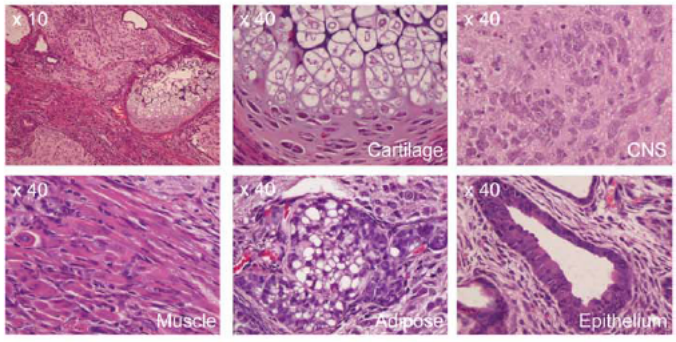
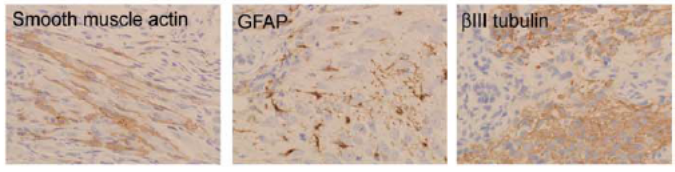
Pluripotency of iPS Cells Derived from MEFs

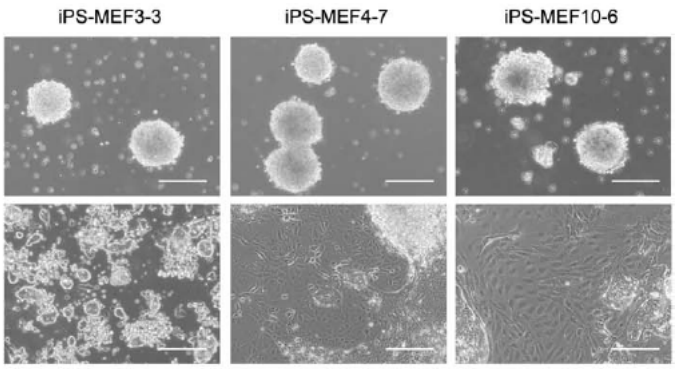
(A) Various tissues present in teratomas derived from iPS-MEF4-7 cells.



(B) Immunostaining confirming differentiation into neural tissues and muscles in teratomas derived from iPS-MEF4-7.



C) In vitro embryoid body formation (upper row) and differentiation (lower row). Scale bars = 200 mm.



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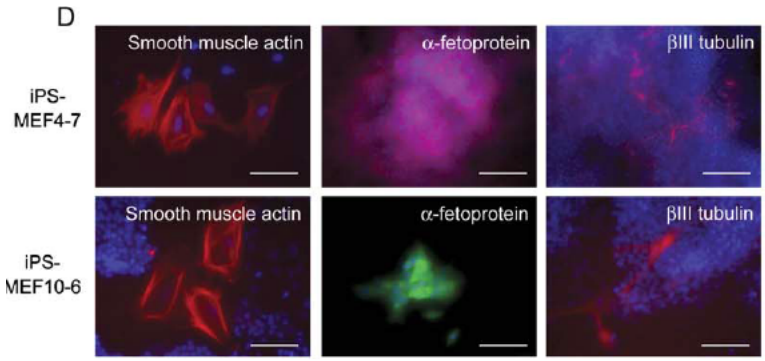
(D) Immunostaining confirming *in vitro* differentiation into all three germ layers. Scale bars = 100 mm. Secondary antibodies were labeled with Cy3 (red), except for a-fetoprotein in iPSMEF10- 6, with which Alexa 488 (green) was used.



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From adult fibroblasts?

We next introduced the four selected factors into tail-tip fibroblasts (TTFs) of four 7-week-old male Fbx15bgeo/bgeo mice on a C57/BL6-129 hybrid background. We obtained 3 G418-resistant colonies, from each of which we could establish iPS cells (iPS-TTF4). We also introduced the four factors into TTFs from a 12-week-old female Fbx15bgeo/bgeo mouse, which also constitutively expressed green fluorescent protein (GFP) from the CAG promoter and had a C57/BL6-129-ICR hybrid background.

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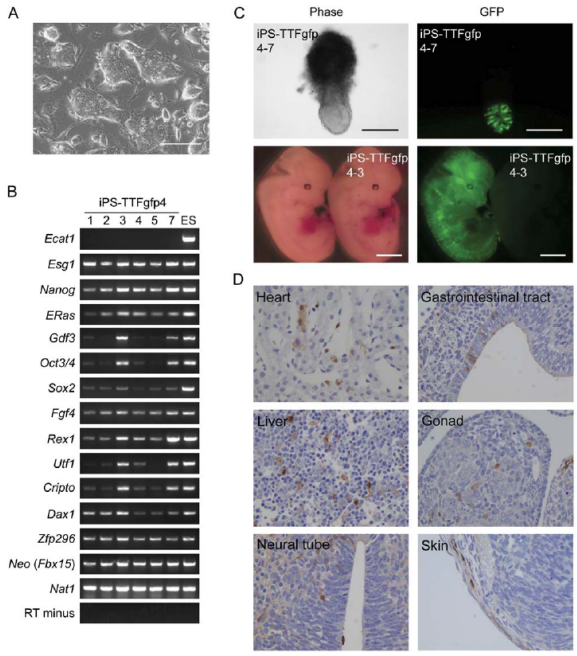
Characterization of iPS Cells Derived from Adult Mouse Tail-Tip Fibroblasts



(C) Contribution of iPS-TTFgfp4-7 and iPS-TTFgfp4-3 cells to mouse embryonic development. iPS cells weremicroinjected into C57/BL6 blastocysts. Embryos were analyzed with a fluorescence microscope at E7.5 (upper panels, iPS-TTFgfp4-7) or E13.5 (lower panels, iPS-TTFgfp4-3). Scale bars = 200 mm (upper panels) and 2 mm (lower panels).

(D) The E13.5 chimeric embryo was sectioned and stained with anti-GFP antibody (brown). Cells were counterstained with eosin (blue).

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Induction of Pluripotency: From Mouse to Human

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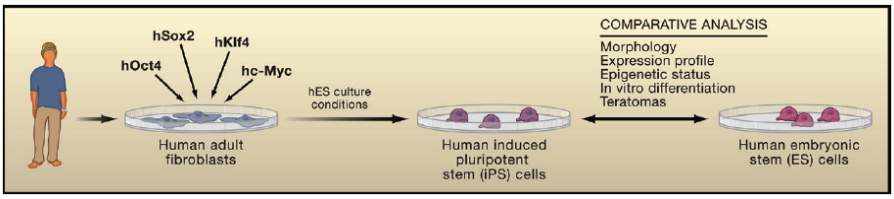

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Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]

Cell *131*, 861–872, November 30, 2007

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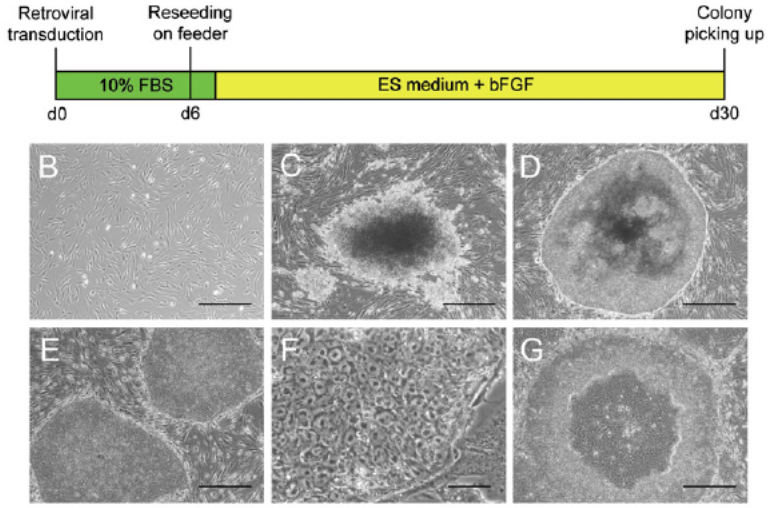
(A) Time schedule of iPS cell generation from adult Human Dermal Fibroblasts (HDF).



(B) Morphology of HDF.
(C) Typical image of non-ES cell-like colony.
(D) Typical image of hES cell-like colony.
(E) Morphology of established iPS cell line at passage number 6 (clone 201B7).
(F) Image of iPS cells with high magnification.
(G) Spontaneously differentiated cells in the center part of human iPS cell colonies.

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Conclusion:

Our study has opened an avenue to generate patient and disease-specific pluripotent stem cells.
Even with the presence of retroviral integration, ***human iPS cells are useful for understanding disease mechanisms, drug screening, and toxicology****. For example, hepatocytes derived from iPS cells with various genetic and disease backgrounds can be utilized in predicting liver toxicity of drug candidates.*
Once the safety issue is overcome*, human iPS cells should also be applicable in* ***regenerative medicine****.*
Human iPS cells, however, are not identical to hES cells: DNA microarray analyses detected differences between the two pluripotent stem cell lines. Further studies are essential to determine whether human iPS cells can replace hES in medical applications.

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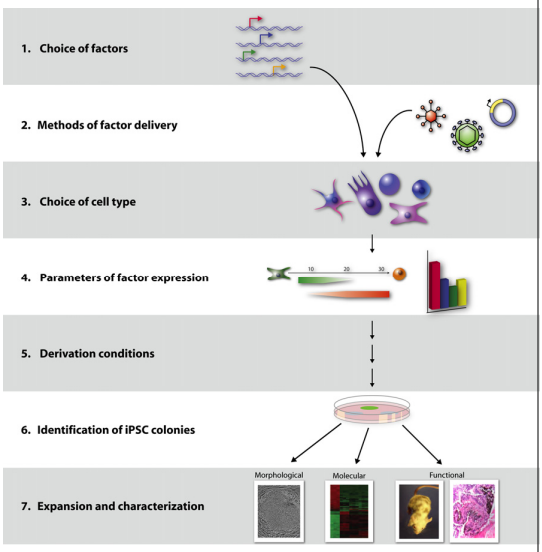
Protocol Review
Cell Stem Cell 3, December 4, 2008 ©2008 Elsevier Inc. 597

Guidelines and Techniques for the Generation of Induced Pluripotent Stem Cells



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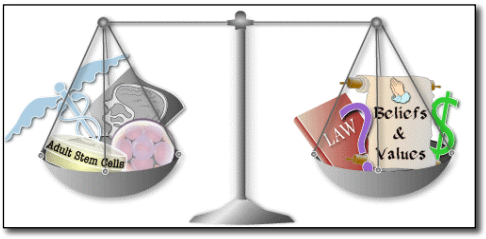
Ethical, legal and social issues.



There are several types of issues to consider as we think about stem cell research.

Ethical issues are those that ask us to consider the potential moral outcomes of stem cell technologies.

Legal issues require researchers and the public to help policymakers decide whether and how stem cell technologies should be regulated by the government.

Social issues involve the impact of stem cell technologies on society as a whole.

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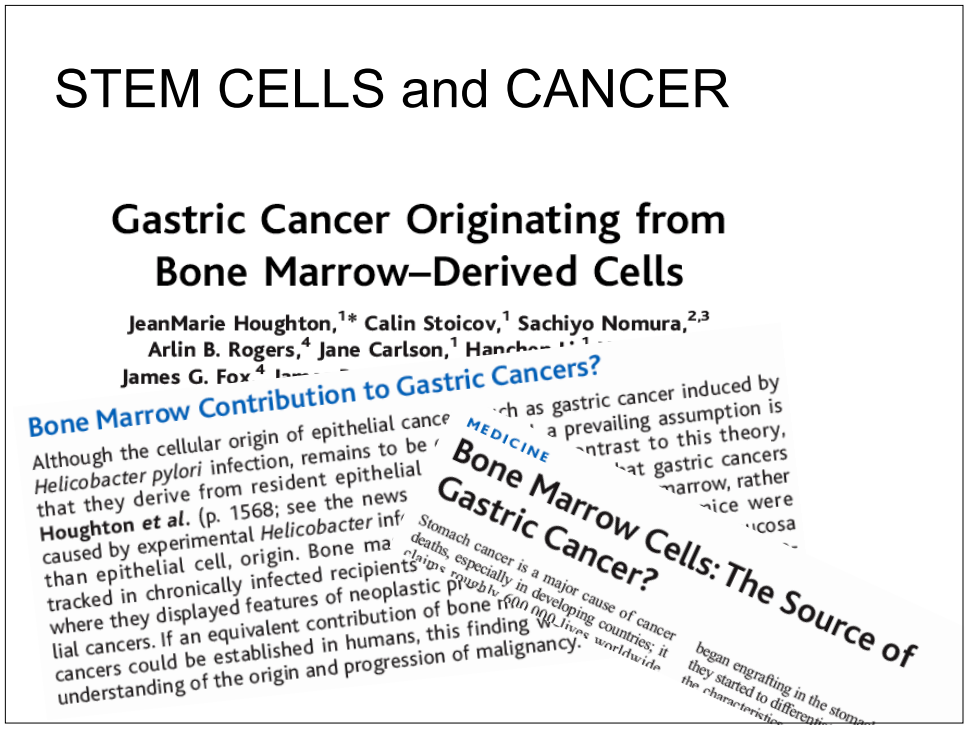
STEM CELLS and CANCER



Gastric Cancer Originating from Bone Marrow–Derived Cells

JeanMarie Houghton,[1]* Calin Stoicov,[1] Sachiyo Nomura,[2,3] Arlin B. Rogers,[4] Jane Carlson,[1] Hanchen Li,[1] James G. Fox,[4]

Bone Marrow Contribution to Gastric Cancers?

Although the cellular origin of epithelial cance... as gastric cancer induced by *Helicobacter pylori* infection, remains to be ... a prevailing assumption is that they derive from resident epithelial ... ntrast to this theory, caused by experimental *Helicobacter* inf... at gastric cancers than epithelial cell, origin. Bone ma... marrow, rather tracked in chronically infected recipients ... nice were where they displayed features of neoplastic pr... icosa lial cancers. If an equivalent contribution of bone ... cancers could be established in humans, this finding ... understanding of the origin and progression of malignancy.

Houghton *et al.* (p. 1568; see the news

MEDICINE

Bone Marrow Cells: The Source of Gastric Cancer?

Stomach cancer is a major cause of cancer deaths, especially in developing countries; it ... began engrafting in the stom... they started to differen...