

embryonické kmeňové bunky a ich využitie

Prečo sa jednotlivé typy buniek líšia
aj keď majú rovnaké gény?

Link 0.5

1

1

Embryonické a adultné kmeňové bunky a ich využitie v liečbe

Výhody

a

Nevýhody

Embryonické SC

“Pluripotentné”

Stabilné. Môžu prejsť
mnohými bunkovými
deleniami.

Je ľahké ich získať, ale je to
za cenu deštrukcie
blastocysty (etická
otázka)

Existuje možnosť imunitnej
reakcie.

Vysoký potenciál tvorby
tumorov

Adultné SC

“Multipotentné”

Menej stabilné. Deliacia kapacita je
limitovaná.

Žiadna etická otázka.

Je ťažké izolovať ich z adultného
tkaniva.

Imunitné odmietnutie veľmi
nepravdepodobné, takmer
absentujúce.

Menší potenciál tvoriť tumory

2

2

Ideálne kmeňové bunky:

- schopnosť diferenciácie na mnoho bunkových typov
- ľahká dostupnosť
- buď špecifické pre dané individuum alebo bez vyvolania imunitnej odpovede
- s veľkým potenciálom proliferácie
- bezpečné
- bez nebezpečenstva tvorby tumorov

Prečo sa jednotlivé typy buniek líšia
aj keď majú rovnaké gény?

[Link 0.5](#)

3

3

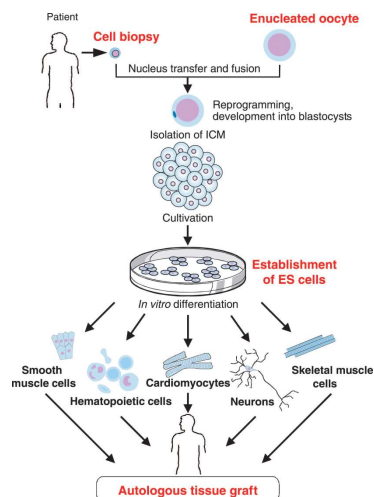
Terapeutické klonovanie

- Využíva transfer jadra na produkciu pluripotentnej embryonickej kmeňovej bunky s rovnakým genómom ako u darcu
- Ak sú takéto bunky prenesené do maternice, vytvorí sa jedinec – ovečka Dolly
- Ak sa takéto bunky kultivujú, vytvoria blastocystu
 - embryonické kmeňové bunky sa potom môžu vyňať z vnútornej časti
 - tieto bunky nevykazujú imunitnú nekompatibilitu s hostiteľom

4

4

Terapeutické klonovanie



1 aký je rozdiel medzi diferencovanou a kmeňovou bunkou?

Link 1

Wobus, A.M. et al. (2005)

<https://www.biointeractive.org/classroom-resources/somatic-cell-nuclear-transfer-footage>

5

Sporná otázka

- Pre tých, čo veria, že embryo je ľudská bytosť od splynutia, je deštrukcia embrya vražda
- Ale takéto kmeňové bunky sú veľmi sľubné v liečbe ochorení...
- Nový prístup: Existuje spôsob, akým by bolo možné získať ľudské embryonálne kmeňové bunky bez deštrukcie ľudského embrya?

6

6

The Induced Pluripotent Stem (iPS) Cell : A Likely Candidate?

- indukované pluripotentné kmeňové bunky

7

7

Criteria of Pluripotent Stem Cells

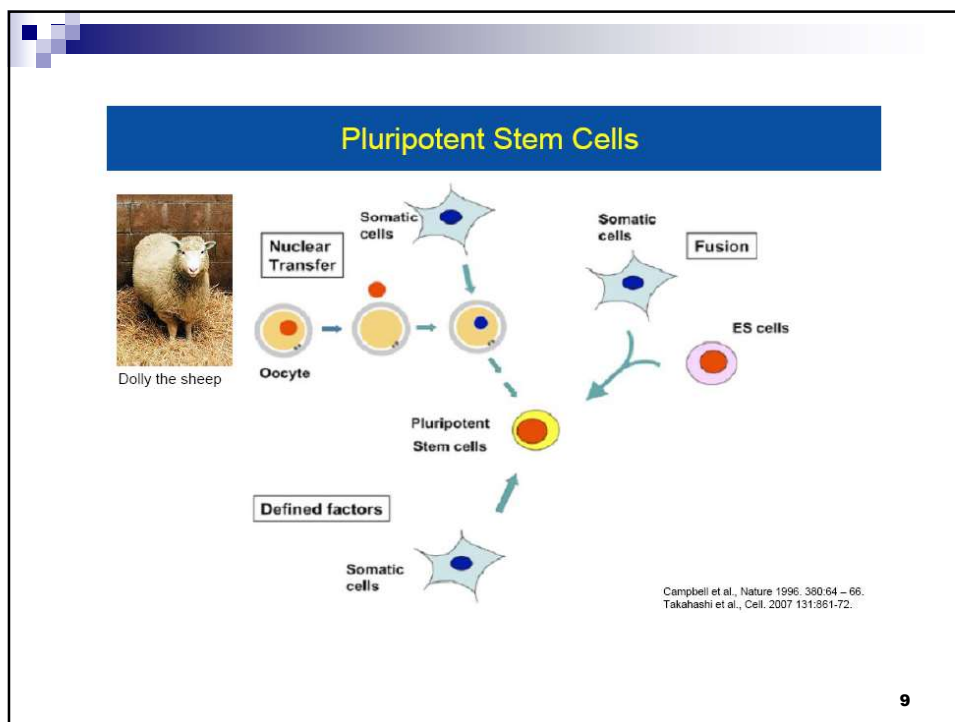
1. Unlimited proliferation *in vitro* and maintenance of normal diploid karyotype
2. Expression of pluripotency markers, such as Oct4, Nanog, stage-specific embryonic antigen (SSEA4), etc.
3. *in vitro* differentiation potential into cells of three germ layers (ectoderm, mesoderm and endoderm).
4. Capacity to form teratoma (composed of tissues from three germ layers), when injected subcutaneously into immune-deficient mice.



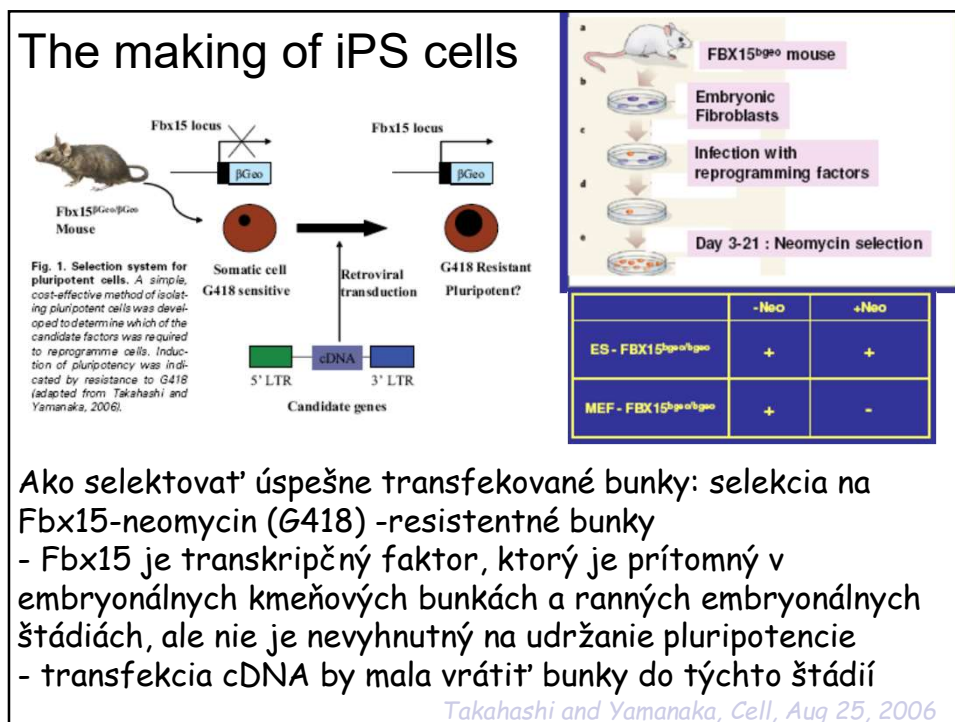
Sci China Ser C-Life Sci, 2009, 52(7): 622-636.

8

8

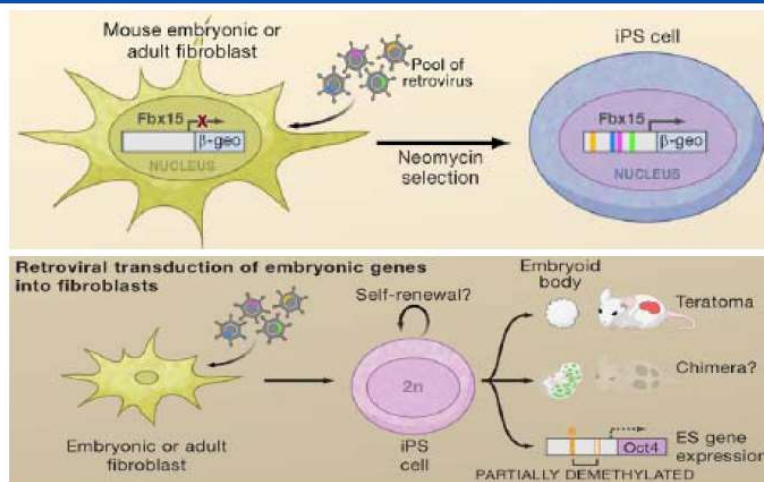


9



10

Induced Pluripotent Stem Cell



Dal by sa laboratóriu vyrobiť živočích, keby sa prenieslo jadro z oocyty do cytoplazmy somatickej bunky?

Yoshida and Eggan, Cell 126, 652-655.

Link 2

11

11

Induced Pluripotent Stem Cell

iPS cells are similar to hESC's regarding:

- Proliferation
- Morphology
- Pluripotency
- Surface markers
- Expression profile
- Epigenetic status
- Differentiation in vitro
- Teratoma formation

Lentiviral transduction of Reprogramming factors: Oct3/4, Sox 2, Klf4 and c-myc



Transduction Efficiency: 0.01-0.1%

Human induced pluripotent stem cells (iPSC's)

Human embryonic stem cells (hESC)

12

12

Pioneering Working on human iPS Cells

Cell. 2007 Nov 30;131(5):861-72.

Induction of pluripotent stem cells from adult human fibroblasts by defined factors.

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S.
(Yamanaka factors: Oct4, Sox2, Klf4, and c-Myc – transcription factors)



Science. 2007 Dec 21;318(5858):1917-20.

Induced pluripotent stem cell lines derived from human somatic cells.

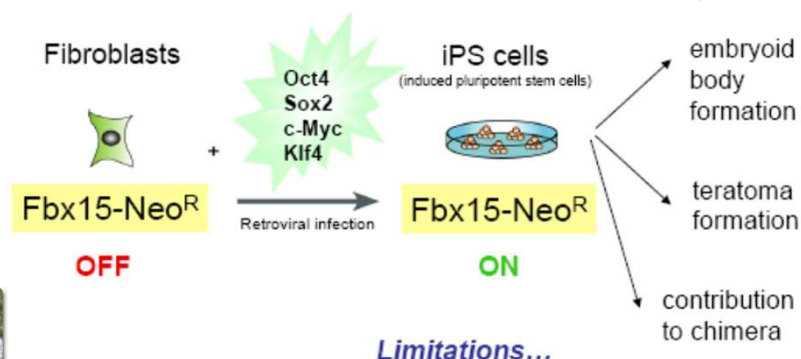
Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA.
(Oct4, Sox2, Nanog, Lin28)

13

13

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

Kazutoshi Takahashi¹ and Shinya Yamanaka^{1,2,*}



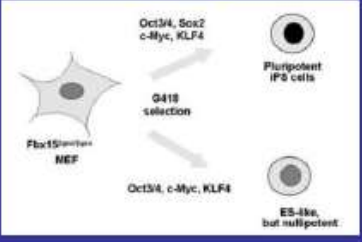
Takahashi & Yamanaka, Cell (2006)

14

14

24 candidate genes expressed in embryonic stem cells:

β -catenin	ERas	Sox2
c-Myc	Dnmt3l	Rex1 (Zfp42)
Stat3	Ecat8	Utf1
Grb2	Gdf3	Tcl1
Ecat1	Sox15	Dppa3(Stella)
Dppa5(Esg1)	Dppa4	Klf4
Fbxo15	Dppa2	Sall4
Nanog	Fthl17	Oct3/4(Pou5f1)



Načo sa pri vytváraní indukovaných kmeňových buniek využila neomycínová rezistencia?

Link 3 Takahashi and Yamanaka, Cell, Aug 25, 2006¹⁵

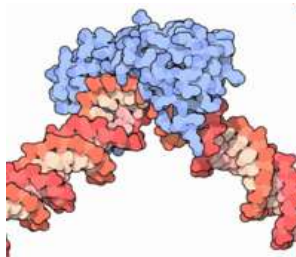
15

Transcription factors for reprogramming

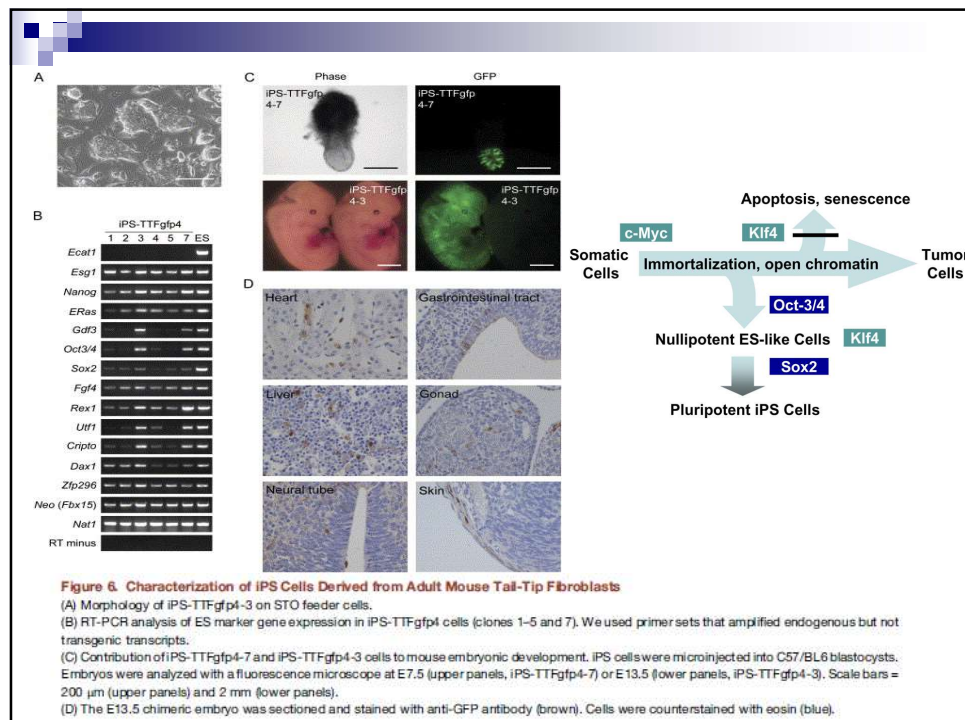
Transcription factors are proteins that bind to DNA and regulate gene expression

Oct3/4 and Sox2: transcription factors that function in maintaining pluripotency in both early embryos and ES cells.

c-Myc and Klf4: transcription factors that modify chromatin structure so that Oct3/4 and Sox2 can bind to their target; proto-oncogenes



16



17

Were these iPS cells identical to the ES cells?

NO

- The transcriptional profile was somewhere between fibroblasts and ES cells
- No live chimeras produced

So these iPS cells were somewhat similar but not identical to ES cells

WHY?

Because fbx15 was selected for. Fbx15 is a factor that is expressed in ES cells but is not essential for the maintenance of pluripotency

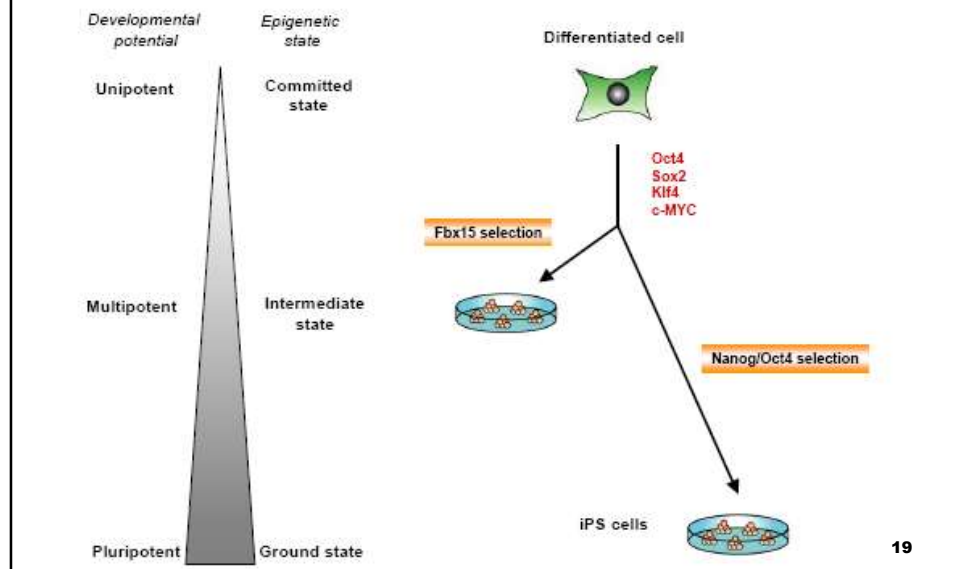
Prečo je potrebné transfekovať somatické bunky transkripčnými faktormi na to aby sa stali kmeňovými?

Link 4

18

18

Proposed explanation for the difference



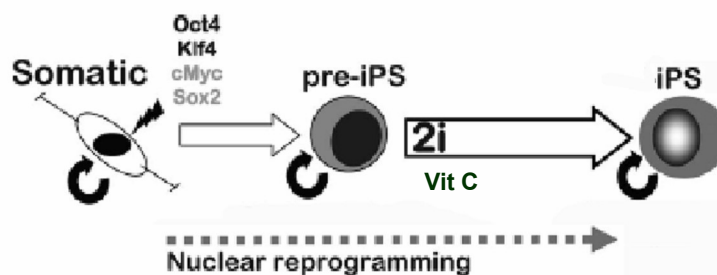
19

Nanog-selected iPS cells

- Expressed all markers and characteristics of ES cells
 - Chimera formation when injected into blastocysts
- but
- 20% of the mice developed tumours

20

Reprogramming: 2-stage process



leukemia inhibitory factor (LIF)
cytokín
potrebný na
propagáciu buniek
a udržanie buniek
nediferencovaných

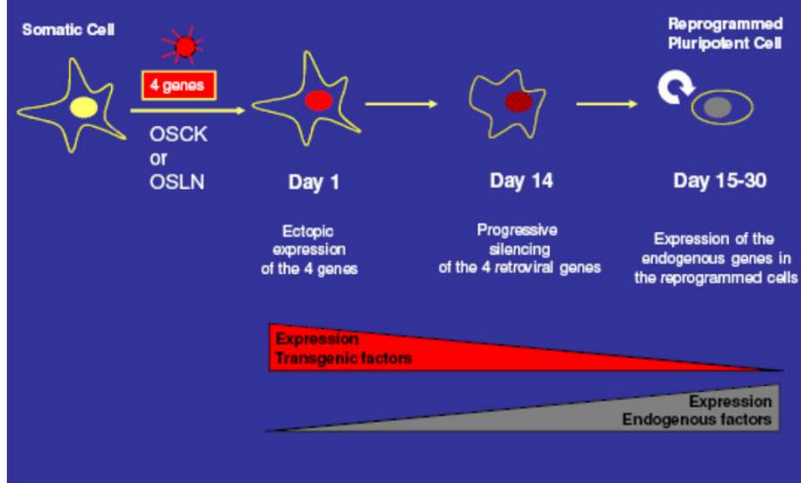
ESC morphology
Some pluripotent
markers
Loss of somatic
markers
LIF unresponsive
No chimeras
Germline incompetent

ESC morphology
All pluripotent
markers
No somatic
markers
LIF responsive
Chimera forming
Germline
competent

21

21

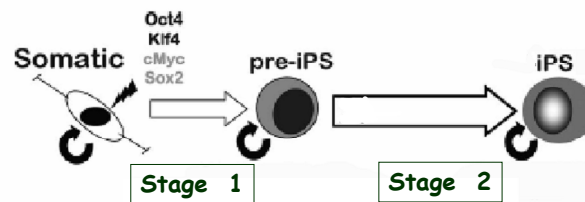
SCHEMATIC OF THE REPROGRAMMING PROCESS



22

22

Induced pluripotency : the two-stage switch



Downregulation of lineage genes
Activation of specific ES genes
Chromatin remodelling

Activation of auto-regulatory loop
Full reactivation of ES cell transcriptional network
Completion of transgene silencing

23

iPS Cells- Starting cells

Mouse

Embryonic fibroblasts
Adult tail fibroblasts
Hepatocytes
Gastric epithelial cell
Pancreatic cell
Neural stem cell
B lymphocyte
Keratinocyte

Human

Skin fibroblast
Keratinocyte
Bone marrow stem cell
Peripheral blood cell

24

24

Efficiency of re-programming is poor

Table 1. Reprogramming of different mouse cell types into iPS cells

Germ layer	Cell type	Efficiency	Selection type	References
Mesoderm	MEFs	0.01-0.1%	Fbx15/Oct4/Nanog	Takahashi and Yamanaka, 2006; Maherali et al., 2007; Okita et al., 2007; Wernig et al., 2007
	MEFs*	2-4%	Nanog	Wernig et al., 2008
	Mature B cell* ¹	3%	Nanog	Hanna et al., 2008
Endoderm	Stomach cells	ND	Fbx15	Aoi et al., 2008
	Liver cells	ND	Fbx15	Aoi et al., 2008
	Intestinal epithelium*	ND	Nanog	Wernig et al., 2008
	β cells	0.1-0.2%	Morphology	Stadtfield et al., 2008
Ectoderm	Neural progenitors	<3.6%	Oct4	Kim et al., 2008; Shi et al., 2008; Eminli et al., 2008
	Keratinocytes*	ND	Nanog	Wernig et al., 2008

*Secondary cells. Differentiated cells derived from iPS cells carrying the four viral transgenes under the control of a doxycycline-inducible promoter.

¹Note that mature B cells required ectopic expression of Cebpa in addition to the four reprogramming factors to produce iPS cells.

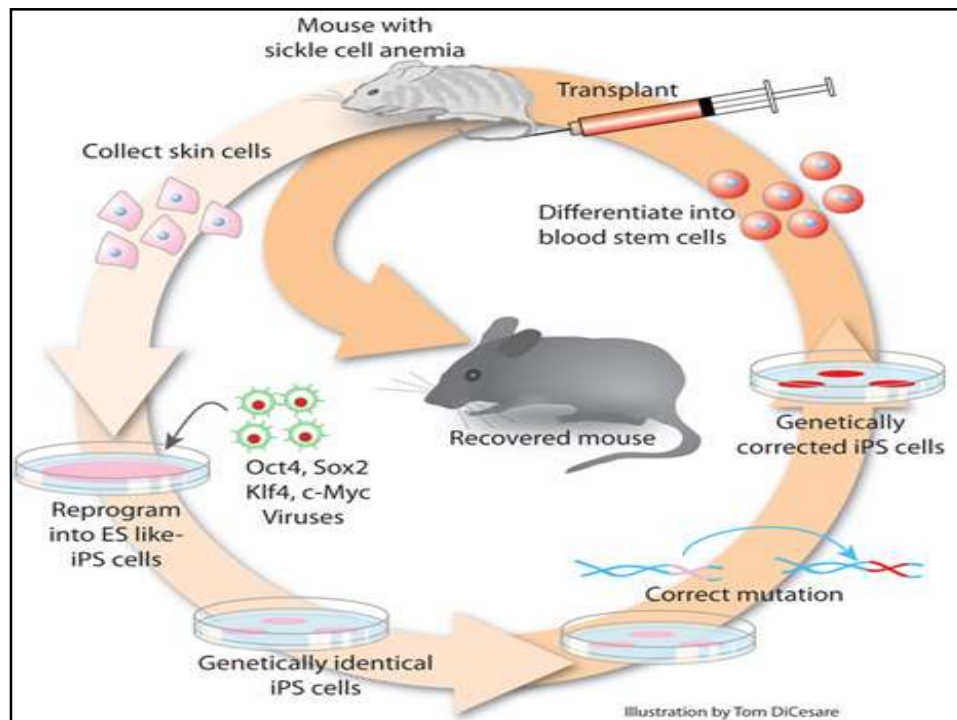
MEFs, murine embryonic fibroblasts. ND, not determined.

Hochdelinger and Plath, 2009

**Prečo bolo potrebné urobiť novú
selekciiu IPSP buniek na expresiu
nanog/oct4?**

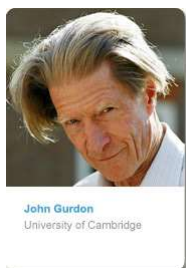
Link 6

25



26

Induced pluripotent stem cells – the science and technology



Albert Lasker Basic Medical Research Award, 2009

Chi-Wei Lu, Ph.D.
Assistant Professor, RWJMS/UMDNJ
Director, Human Embryonic Stem Cell Facility

Ako dlho je
stabilná
transfekcia
exogénnym
transkripčným
mi faktormi?

[Link 7](#)

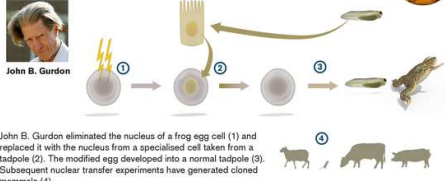
27

The Nobel Prize in Physiology or Medicine 2012



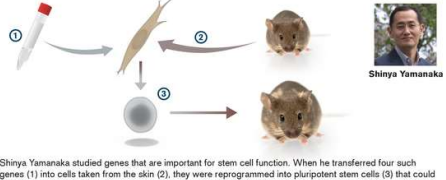
1962

John B. Gurdon



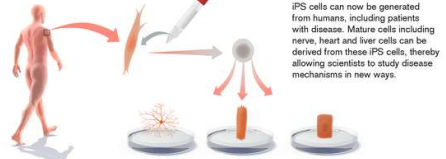
John B. Gurdon eliminated the nucleus of a frog egg cell (1) and replaced it with the nucleus from a specialised cell taken from a tadpole (2). The modified egg developed into a normal tadpole (3). Subsequent nuclear transfer experiments have generated cloned mammals (4).

Shinya Yamanaka



Shinya Yamanaka studied genes that are important for stem cell function. When he transferred four such genes (1) into cells taken from the skin (2), they were reprogrammed into pluripotent stem cells (3) that could develop into all cell types of an adult mouse. He named these cells induced pluripotent stem (iPS) cells.

iPS cells can now be generated from humans, including patients with disease. Mature cells including nerve, heart and liver cells can be derived from these iPS cells, thereby allowing scientists to study disease mechanisms in new ways.



© 2012 The Nobel Committee for Physiology or Medicine
The Nobel Prize® and the Nobel Prize® medal design mark are registered trademarks of the Nobel Foundation

Illustration and layout: Mattias Karlén

<http://www.leica-microsystems.com/science-lab/nobel-prize-2012-in-physiology-or-medicine-for-stem-cell-research/>

28

The induced pluripotency phenomena in human cells

- Takahashi and Yamanaka (Cell, 2007) :
retroviral vector (pLAT-A) with Oct4, Sox2, Klf4 and c-Myc
- Yu and Thomson (Science, 2007):
lentiviral vector (pSIN) with Oct4, Nanog, Sox2 and Lin28
- Park and Daley (Nature 2008):
retroviral vectors (pMIG): Yamanaka factors with SV40T and hTert
- Lowry and Plath (PNAS 2008) :
retroviral vectors (pMX): Yamanaka factors

Colonies can be identified after 14 days, select by colony morphology.

29

29

iPS cell reprogramming: Problems

Use of viral vectors for induction

Low efficiency of reprogramming

Risk of tumour formation

Efficient differentiation protocols required

30

Induction of iPS Cells by Proteins

Cell Stem Cell
Brief Report



Generation of Induced Pluripotent Stem Cells Using Recombinant Proteins

Hongyan Zhou,¹ Shili Wu,^{4,7} Jin Young Joo,^{5,7} Saiyong Zhu,¹ Dong Wook Han,⁶ Tongxiang Lin,¹ Sunia Trauger,^{2,3} Geoffrey Bian,⁴ Susan Yao,⁴ Yong Zhu,⁴ Gary Siuzdak,^{2,3} Hans R. Schöler,⁵ Lingxun Duan,⁶ and Sheng Ding^{1,*}

¹Department of Chemistry

²Department of Molecular Biology

³Center for Mass Spectrometry

The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

⁴ProteomTech, Inc., 3505 Cadillac Avenue, Suite F7, Costa Mesa, CA 92626, USA

⁵Department of Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, Röntgenstrasse 20,

Münster 48149, Germany

⁶LD Biopharma Inc., Sandown Way, San Diego, CA 92130, USA

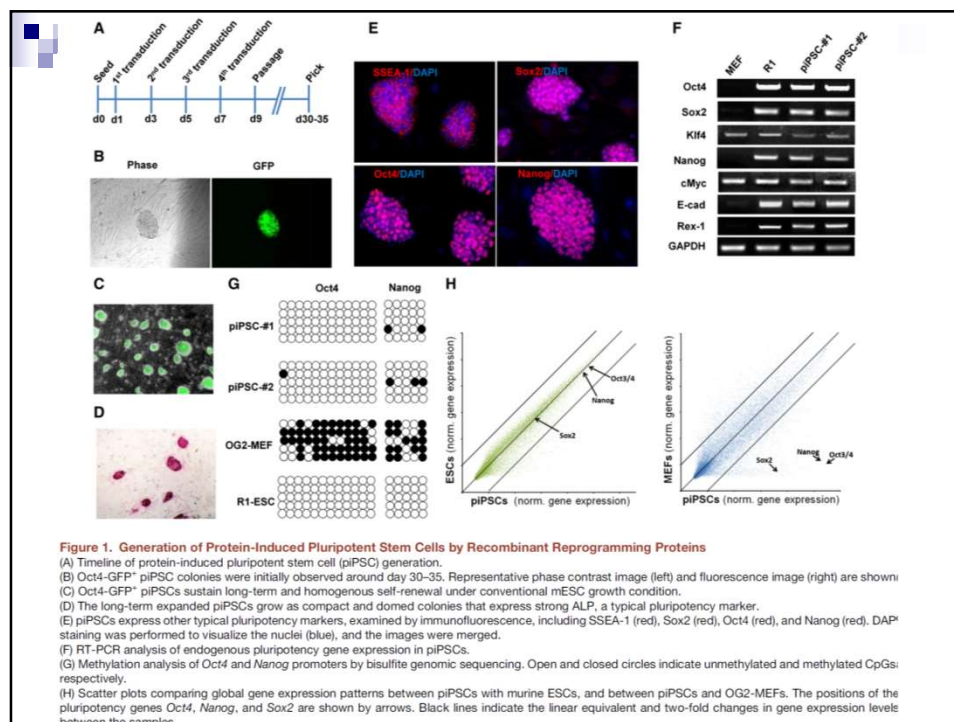
⁷These authors contributed equally to this work

*Correspondence: sdg@scripps.edu

DOI: 10.1016/j.stem.2009.04.005

31

31



32



Induction of iPS Cells by Proteins

Cell Stem Cell
Brief Report

Generation of Human Induced Pluripotent Stem Cells by Direct Delivery of Reprogramming Proteins

Dohoon Kim,^{1,5} Chun-Hyung Kim,^{1,5} Jung-Il Moon,¹ Young-Gie Chung,³ Mi-Yoon Chang,¹ Baek-Soo Han,¹
Sanghyeok Ko,¹ Eungi Yang,¹ Kiwang Yui Cha,⁴ Robert Lanza,^{3,*} and Kwang-Soo Kim^{1,2,4,*}

¹Molecular Neurobiology Laboratory, Department of Psychiatry and McLean Hospital, Harvard Medical School
²Harvard Stem Cell Institute
115 Mill Street, Belmont, MA 02478, USA
³Stem Cell and Regenerative Medicine International, 381 Plantation Street, Worcester, MA 01605, USA
⁴CHA Stem Cell Institute, CHA University, 606-16 Yoksam 1-dong, Gangnam-gu, Korea
⁵These authors contributed equally to this work
*Correspondence: rlanza@advancedcell.com (R.L.), kskim@mclean.harvard.edu (K.-S.K.)
DOI 10.1016/j.stem.2009.05.005

Transduction efficiency is even less 0.001% compared to 0.01% by lentivirus

33

33

Conclusions

- Reprogramming patient cells into a **pluripotent state** provides the best matching and the most abundant source for tissue regeneration.
- Among all different methods, the most achievable is **direct reprogramming**, by introducing pluripotency associated transcription factors into primary tissue culture.

34

34

... d'akujem za pozornosť

35

iPS cells generation in patient fibroblasts

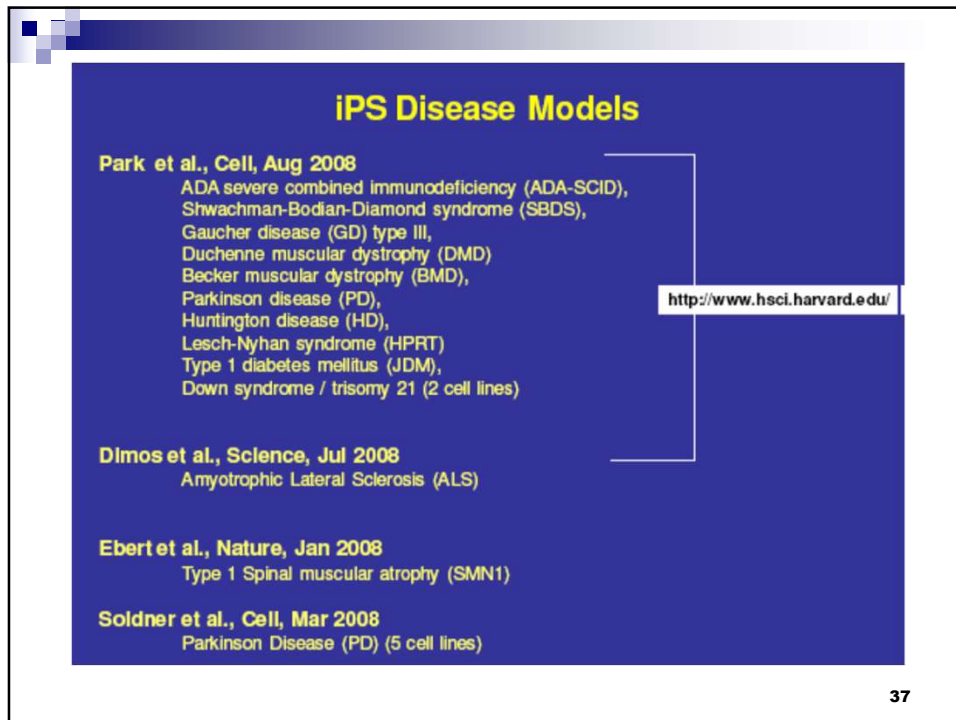
- Parkinson's disease (Wernig and Jaenisch, 2008, Maehr and Melton PNAS 2009).
- Amyopathic Lateral Sclerosis, (Dimos and Eggan Science 2008)
- Type I diabetes (Maehr and Melton PNAS 2009)
- ADA-SCID, SBDS, Gaucher disease, Duchenne and Becker Muscular dystrophie, Huntington disease, JDM, Down syndrome, Lesch-Nyhan syndrome. (Park and Daley Cell 2008).

iPS cells generation from other cell types

- Blood cells (Loh and Daley 2009). **B-cells** (Hanna and Jaenisch Cell 2008)
- Blood stem cells (Eminli and Hochedlinger Nat Genet 2009)
- Pancreatic β -cells (Stadtfeld and Hochedlinger Cell Stem Cell 2008)
- Hepatic and gastric endoderm (Aoi and Yamanaka Science 2008)
- Neural stem cells (Kim and Scholer, Nature 2008)

36

36



iPS Disease Models

Park et al., Cell, Aug 2008
 ADA severe combined immunodeficiency (ADA-SCID),
 Shwachman-Bodian-Diamond syndrome (SBDS),
 Gaucher disease (GD) type III,
 Duchenne muscular dystrophy (DMD)
 Becker muscular dystrophy (BMD),
 Parkinson disease (PD),
 Huntington disease (HD),
 Lesch-Nyhan syndrome (HPRT)
 Type 1 diabetes mellitus (JDM),
 Down syndrome / trisomy 21 (2 cell lines)

Dimos et al., Science, Jul 2008
 Amyotrophic Lateral Sclerosis (ALS)

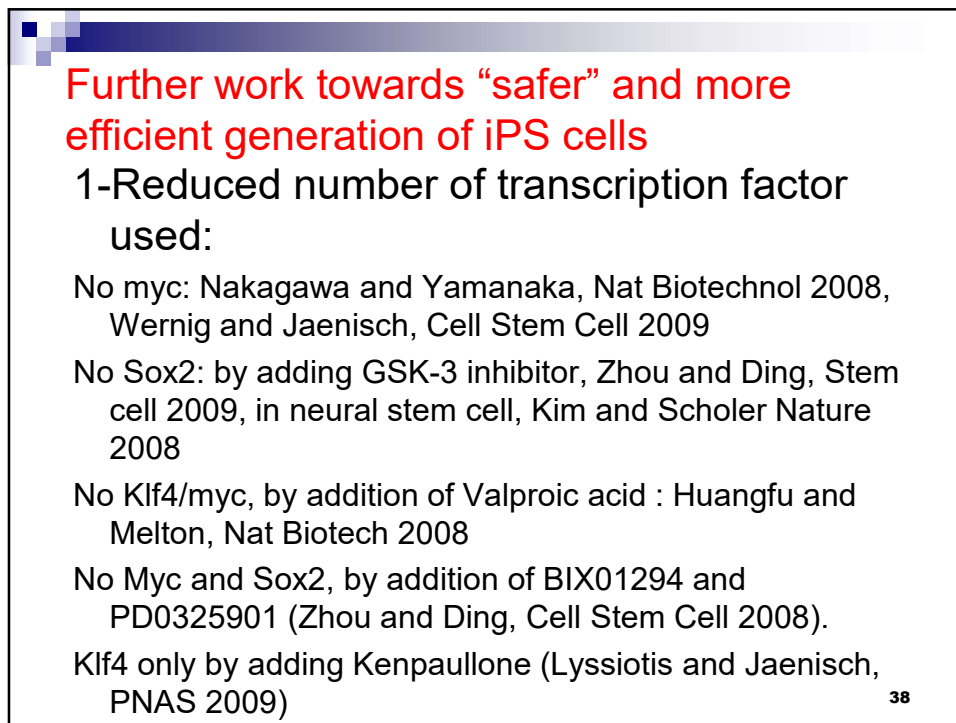
Ebert et al., Nature, Jan 2008
 Type 1 Spinal muscular atrophy (SMN1)

Soldner et al., Cell, Mar 2008
 Parkinson Disease (PD) (5 cell lines)

<http://www.hsci.harvard.edu/>

37

37



Further work towards "safer" and more efficient generation of iPS cells

1-Reduced number of transcription factor used:

No myc: Nakagawa and Yamanaka, Nat Biotechnol 2008,
 Wernig and Jaenisch, Cell Stem Cell 2009

No Sox2: by adding GSK-3 inhibitor, Zhou and Ding, Stem cell 2009, in neural stem cell, Kim and Scholer Nature 2008

No Klf4/myc, by addition of Valproic acid : Huangfu and Melton, Nat Biotech 2008

No Myc and Sox2, by addition of BIX01294 and PD0325901 (Zhou and Ding, Cell Stem Cell 2008).

Klf4 only by adding Kenpauillone (Lyssiotis and Jaenisch, PNAS 2009)

38

38

2-Specific pathways:

TGF- β inhibitor replaces Sox2 and cMyc and induce Nanog
(Maherali and Hochedlinger, Curr Biol 2009, Ichida and
Eggan 2009)

p53 inhibition augments iPS efficiency (Hong and
Yamanaka, Nature 2009, ^{Park and Daley Cell 2008} Utikal and Hochedlinger Nature
2009, Marion and Blastco Nature 2009, Li and Serrano
Nature 2009, Kawamura and Belmonte 2009)

Hypoxia stimulates iPS generation – Yoshida and
Yamanaka Cell Stem Cell 2009

Wnt signaling stimulates reprogramming efficiency
(Marsonm, Jaenisch Cell Stem Cell 2008)

39

39

... ďakujem za pozornost'

40