
SCIENTIFIC REVOLUTION
• Sekvenování genomu a bioinformatika
• Kmenové buňky vytvořené genetickou manipulací
• Materiálové a tkáňové inženýrství
• Editace genomu

Nobelova cena za fyziologii a medicínu 2012

The Nobel Prize in Physiology or Medicine 2012
Sir John B. Gurdon, Shinya Yamanaka

The Nobel Prize in Physiology or Medicine 2012

Sir John B. Gurdon

Shinya Yamanaka

Photo: Creative Commons Attr. 2.0 Generic license

Sir John B. Gurdon

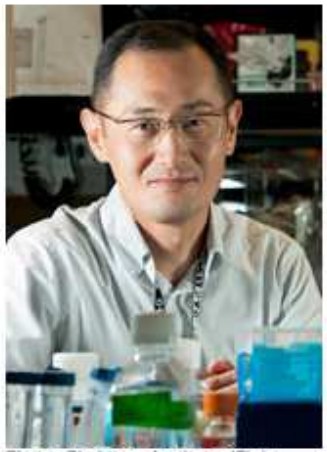
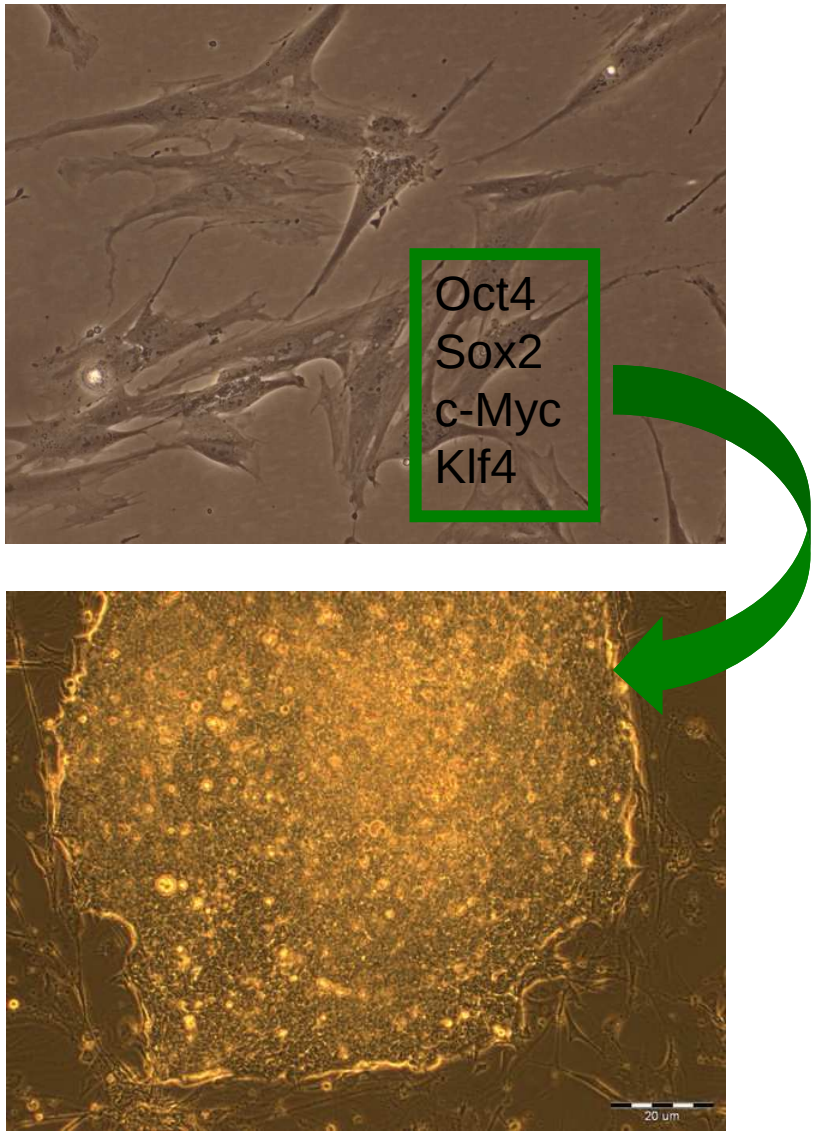


Photo: Gladstone Institutes/Chris Goodfellow

Shinya Yamanaka

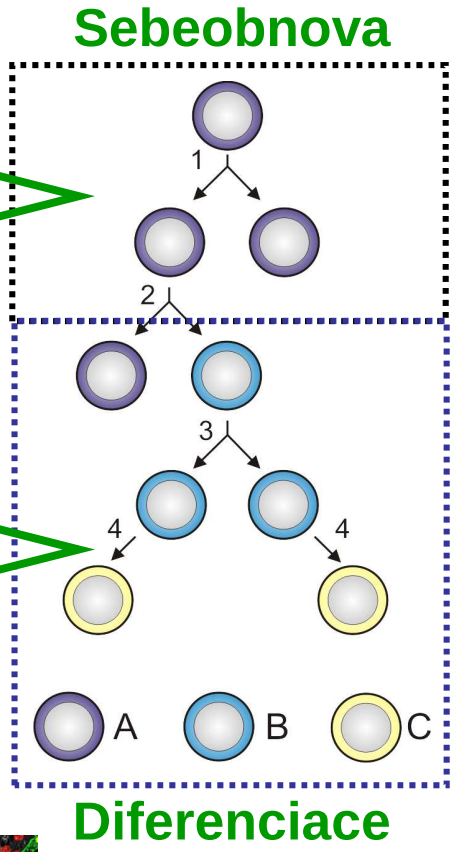
The Nobel Prize in Physiology or Medicine 2012 was awarded jointly to Sir John B. Gurdon and Shinya Yamanaka *"for the discovery that mature cells can be reprogrammed to become pluripotent"*



Kmenové buňky: kriteria a definice

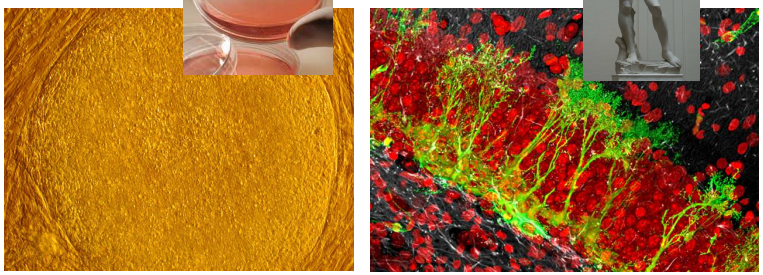
Schopnost vytvářet vlastní kopie

Schopnost měnit vlastnosti a funkčně se specializovat



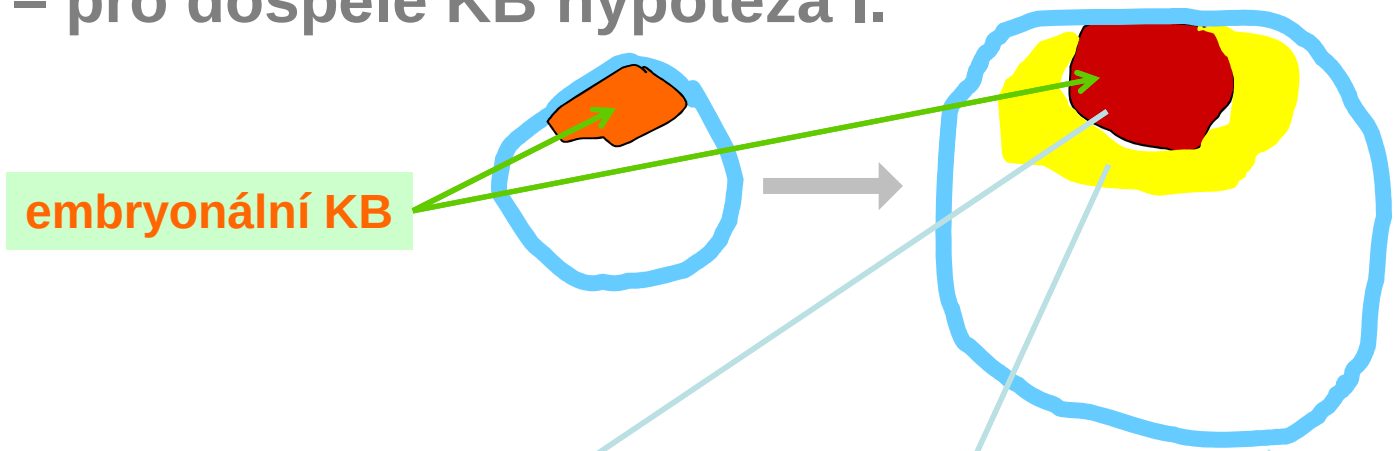
Klonální kapacita
- Symetrické dělení
- Asymetrické dělení

potence:
Toti
Pluri
Multi
Oligo
Uni



- **Embryonální kmenové buňky**
- **“Dospělé”kmenové buňky**
- **Indukované pluripotentní kmenové buňky**
- ~~**Stresem indukované (STAP) buňky**~~

Původ a vývojová ontogeneze kmenových buněk (KB) – pro dospělé KB hypotéza I.



embryonální KB

Primitivní ektoderm/epiblast

Primordiální zárodečné buňky
- **gamety**

Ektoderm
- **nervová tkáň – neurální KB**
- **kůže – kožní KB**

Mesoderm
- **kostní dřeň a krev – hematopoetické a mesenchymální KB**
- **svaly a kosti – tkáňově specifické KB**

Entoderm
- **plíce, játra, pankreas – orgánově specifické KB**
- **jícen, žaludek, střevo – intestinální KB**

Primitivní entoderm

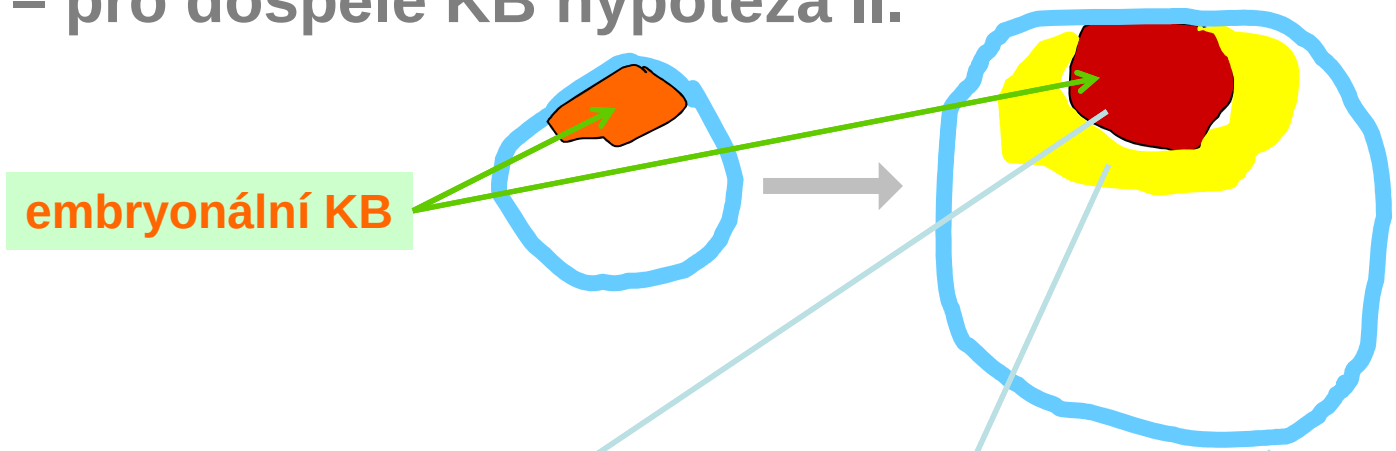
Viscerální entoderm
Parietální entoderm

Trofektoderm

Polární trofektoderm
Extraembryonální ektoderm
Choriový ektoderm
Trofoblast placenty
} placenta

Murální trofektoderm
Ektoplacentální konus
Obří buňky trofoblastu
} parietální žloutkový váček

Původ a vývojová ontogeneze kmenových buněk (KB) – pro dospělé KB hypotéza II.



embryonální KB

Primitivní ektoderm/epiblast

Primitivní entoderm

Trofektoderm

Primordiální zárodečné buňky
- **gamety**

Ektoderm
- **nervová tkáň**
- **kůže**

Mesoderm
- **kostní dřeň a krev**
- **svaly a kosti**

Entoderm
- **plíce, játra, pankreas**
- **jícen, žaludek, střevo**

Multipotentní KB
- **neurální KB**
- **kožní KB**
- **hematopoetické KB**
- **mesenchymální KB**
- **intestinální KB**
- **tkáňově specifické KB**
- **orgánově specifické KB**

Viscerální entoderm
Parietální entoderm

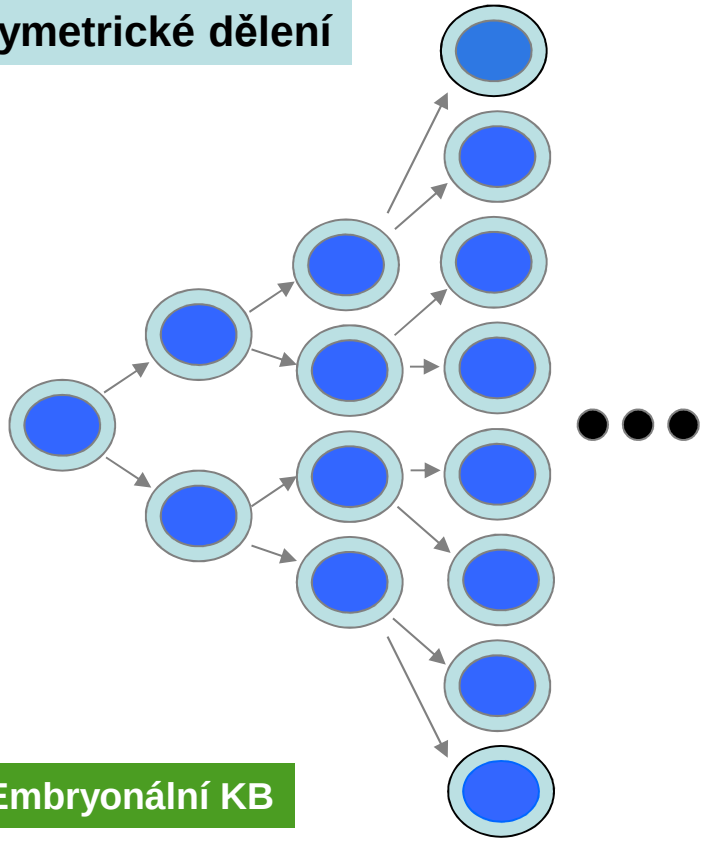
Polární trofektoderm
Extraembryonální ektoderm
Choriový ektoderm
Trofoblast placenty
} placenta

Murální trofektoderm
Ektoplacentální konus
Obří buňky trofoblastu
} parietální žloutkový váček

Kmenové buňky se sebeobnovují, množí

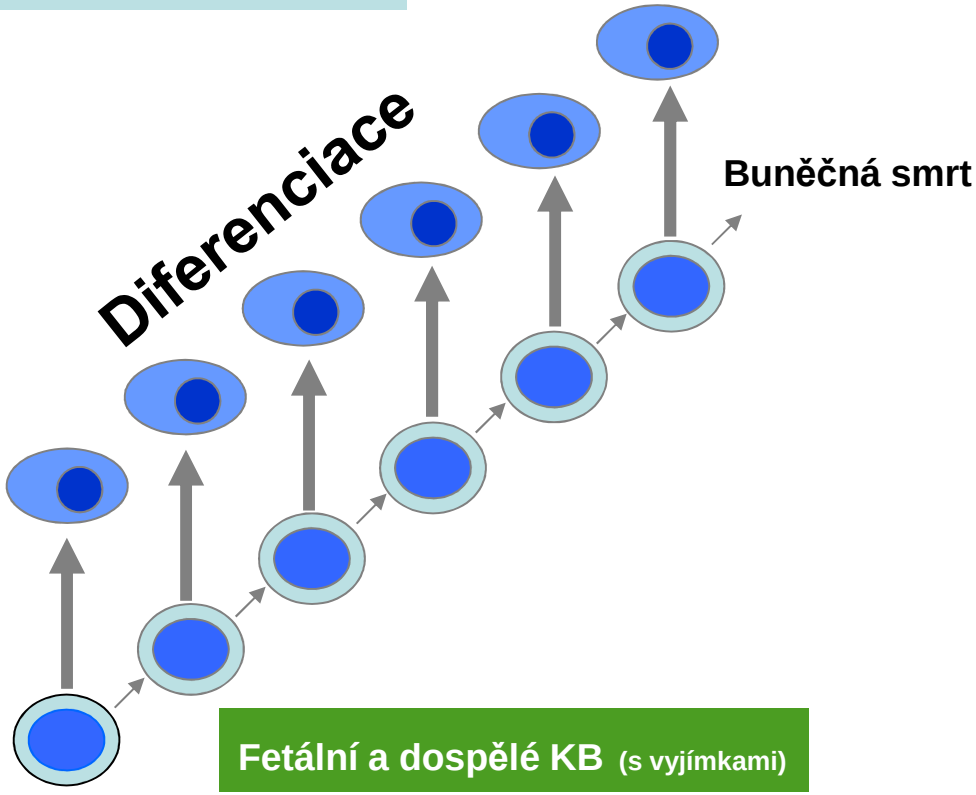
Sebeobnova = tzv. self-renewal; nejdůležitější vlastnost kmenových buněk; schopnost vytvořit identické dceřiné buňky

Symetrické dělení



Embryonální KB

Asymetrické dělení



Fetální a dospělé KB (s vyjímkami)

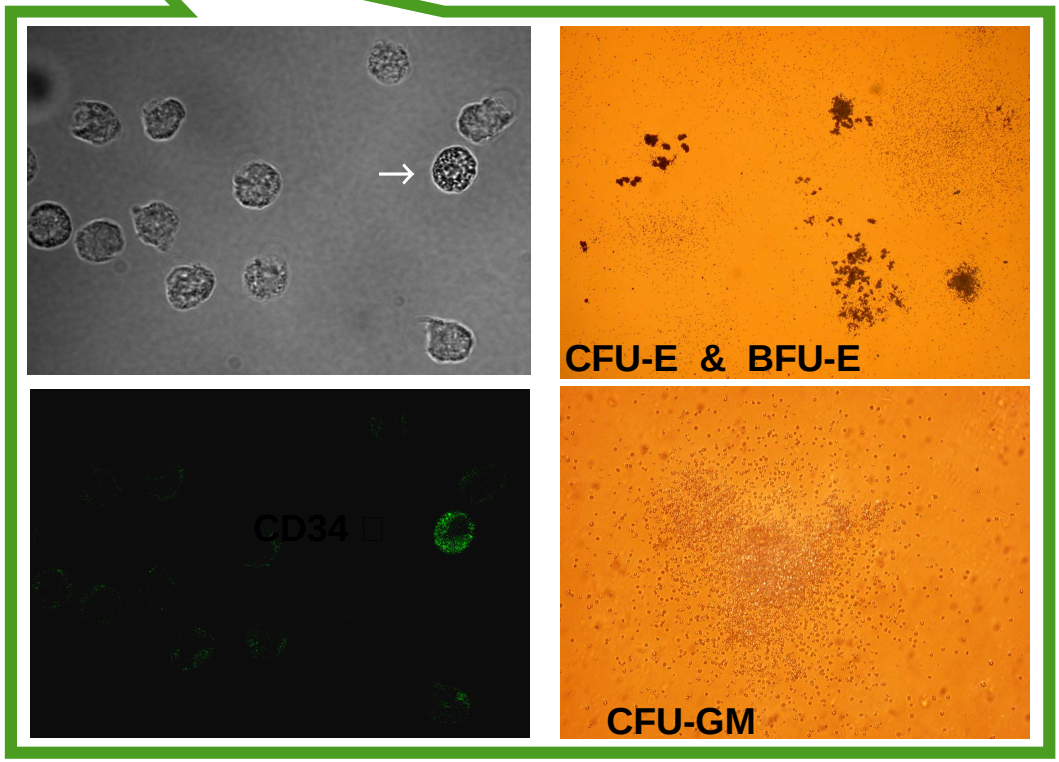
Kombinace obou mechanismů = neurální KB !!!

Kmenové buňky mají obecně velké jádro, tzv. otevřený chromatin a málo cytoplazmy

.... a diferencují a regenerují tkáně orgány

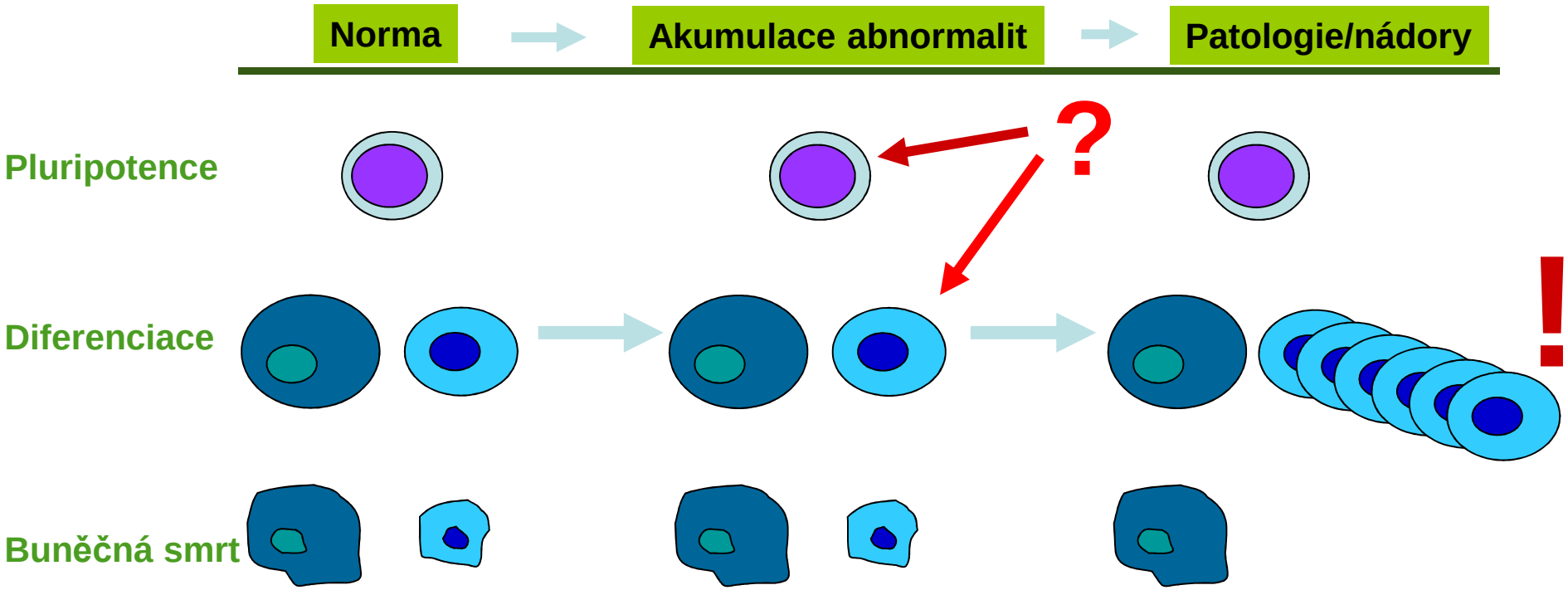
totipotence ➡ pluripotence ➡ multipotence ➡ oligopotence ➡ unipotence

zygota | Embryonální KB | Hematopoetické KB | Gastrointestinální KB | KB prostaty



Příklady

Pochopení molekulárních mechanismů, které řídí sebeobnovu a diferenciaci normálních KB představuje klíč k pochopení vzniku mnoha nádorových onemocnění !!!

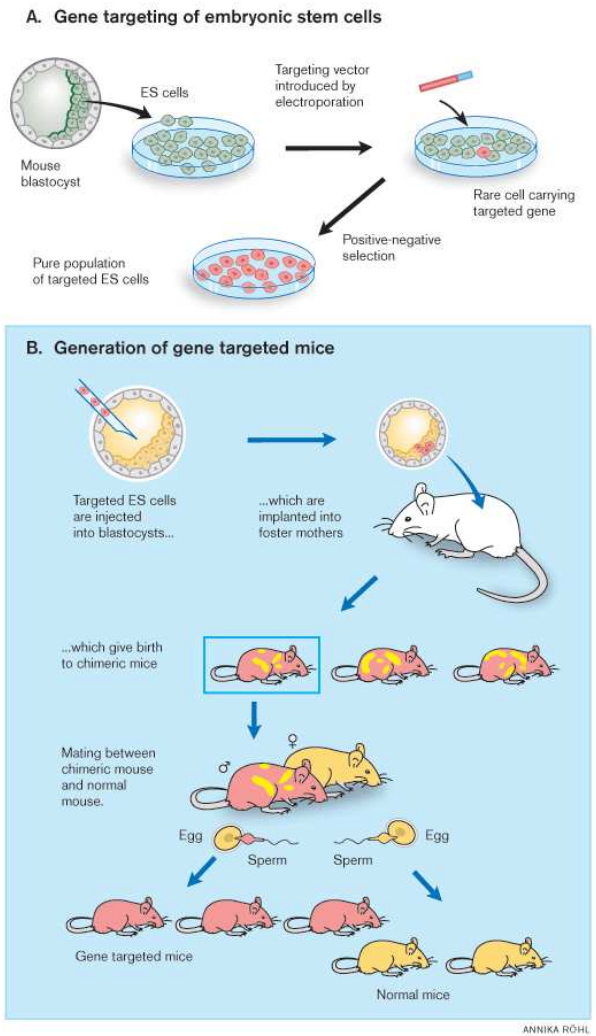


+ Hypotéza nádorových kmenových buněk

Nádory obsahují “mutované” KB, které jsou schopné re-populovat nádor nebo mohou být dokonce jeho počátkem - *např. nádory tlustého střeva a nádory mozku*

Nobelova cena za fyziologii a medicínu 2007

Vývoj technik pro produkci tzv. “knockout” myší prostřednictvím embryonálních kmenových buněk jako nosičů genů – možnost vytvořit živý organismus s požadovanou mutací v každé buňce těla

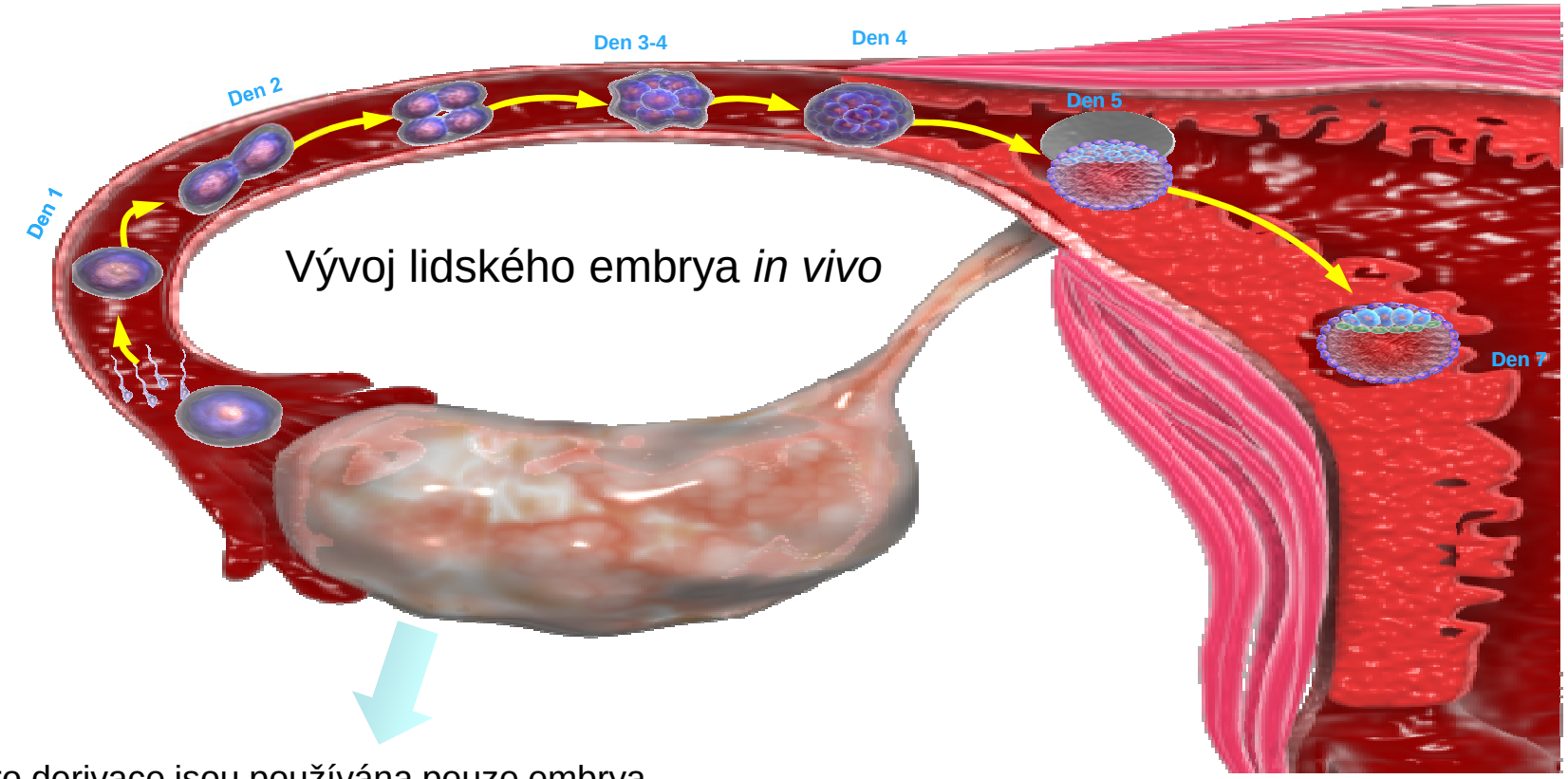


Sir Martin Evans

Mario R. Capecchi

Oliver Smithies

Etické a legislativní aspekty derivace kmenových buněk z lidských embryí

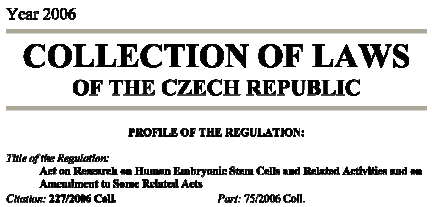


Pro derivace jsou používána pouze embrya získaná po fertilizaci a vývoji *in vitro*

&

- Embrya nepoužitelná pro léčbu
- Informovaný souhlas dárců
- Souhlas etické komise
- Dobrý vědecký důvod
- Potenciál pro medicínu

\+


Year 2006

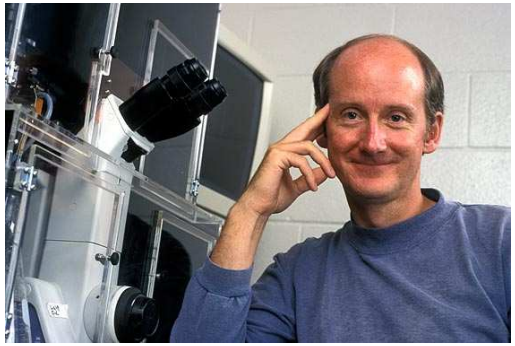
COLLECTION OF LAWS
OF THE CZECH REPUBLIC

PROFILE OF THE REGULATION:

Title of the Regulation:
Act on Research on Human Embryonic Stem Cells and Related Activities and on Amendment to Some Related Acts
Citation: 227/2006 Coll. *Part:* 75/2006 Coll.

=

Embryonální kmenové buňky z lidských embryí v Madisonu a Brně



1998 - James Thomson

www.sciencemag.org SCIENCE VOL 282 6 NOVEMBER 1998

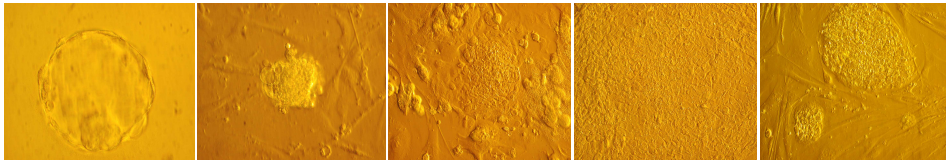
REPORTS

Embryonic Stem Cell Lines Derived from Human Blastocysts

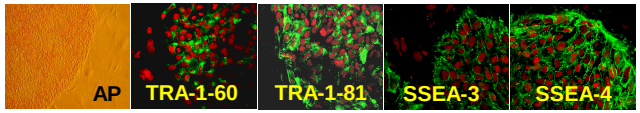
James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, Jeffrey M. Jones

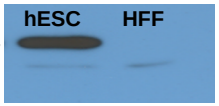
2002/3 – Aleš Hampl & Petr Dvořák

Martina Vodinská
Táňa Košková
Klára Koudelková
Iveta Peterková



Derivace a charakterizace linií





Czech stem-cell work heightens calls for EU ruling

Alison Abbott

Czech scientists say they have derived three human embryonic stem-cell lines from spare embryos stored at an *in vitro* fertilization clinic in Brno.

This makes the Czech Republic the first of the eastern European countries poised to join the European Union (EU) to move into this controversial research area.

It also adds to pressure on the EU to decide whether to fund research on newly derived stem-cell lines. This research is allowed under strict ethical supervision in some EU countries, such as Britain and Sweden, but is banned in others, including Germany and Italy. Last week the Spanish government changed sides and approved a proposed law to allow the production of cell lines from spare embryos for research.

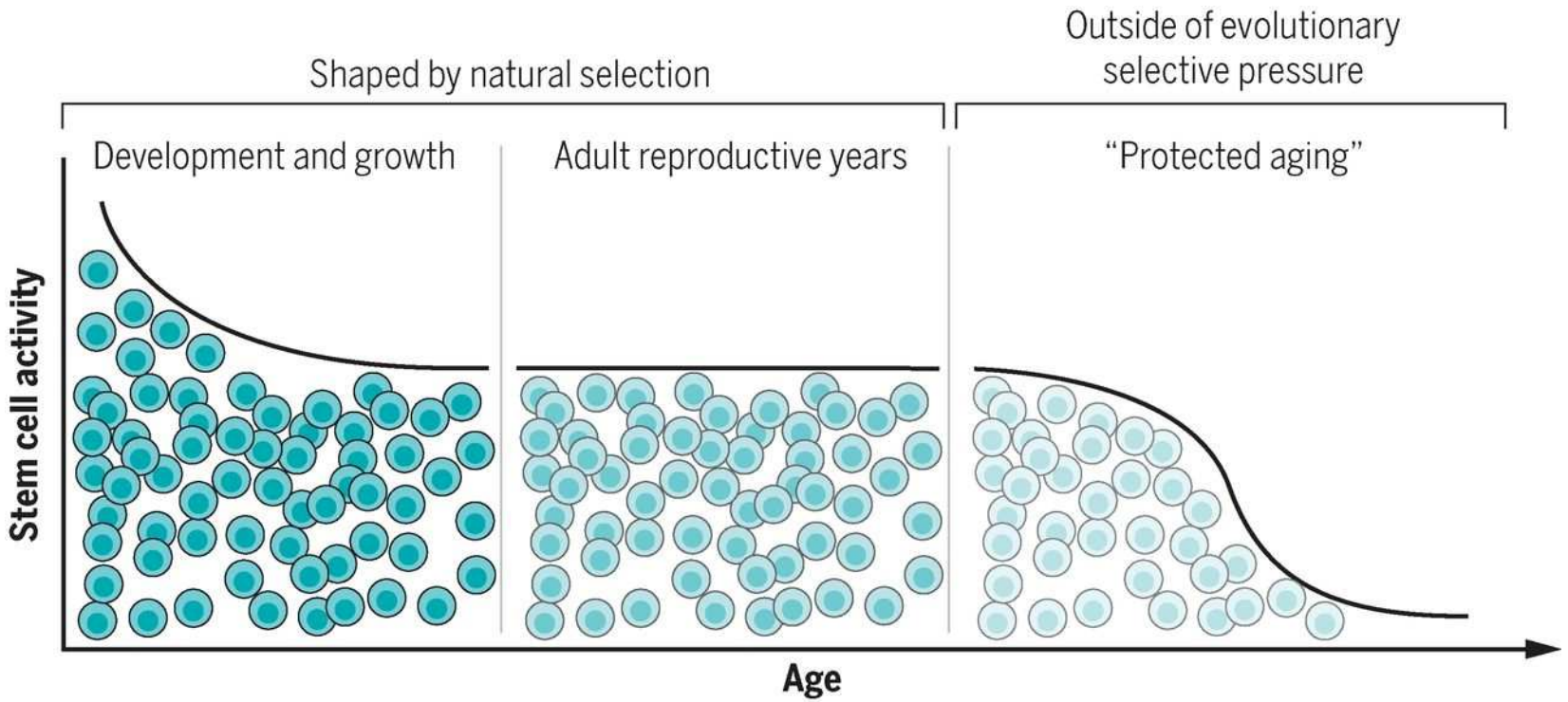
The Czech Republic has no law controlling human embryonic stem-cell research. But Eva Syková, head of the Centre for Cell Therapy and Tissue Repair at Charles University in Prague, who developed the three cell lines together with colleagues at the Mendel University of Agriculture and Forestry in Brno, says they are working to high ethical standards. They received informed consent from donor couples undergoing *in vitro* fertilization, she points out.

The scientists are now characterizing the lines, and plan to study the cells' potential to develop into differentiated cells such as neurons, which they believe could have therapeutic potential. They presented their results at a meeting in Prague last month.

NATURE | VOL 424 | 7 AUGUST 2003 | www.nature.com/nature

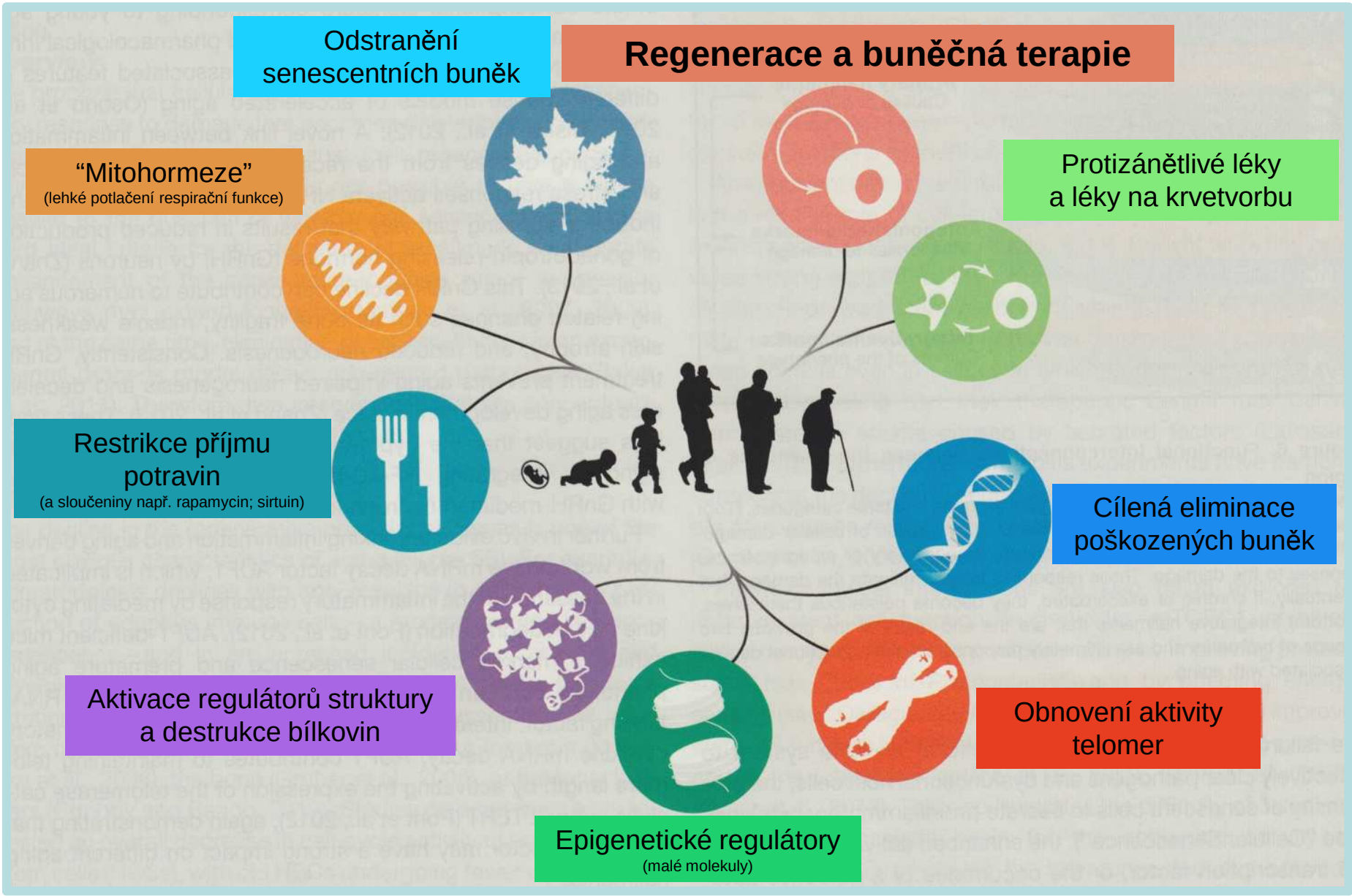
Proč nežijeme věčně, když kmenové buňky…?

Model of stem cell use over the life span



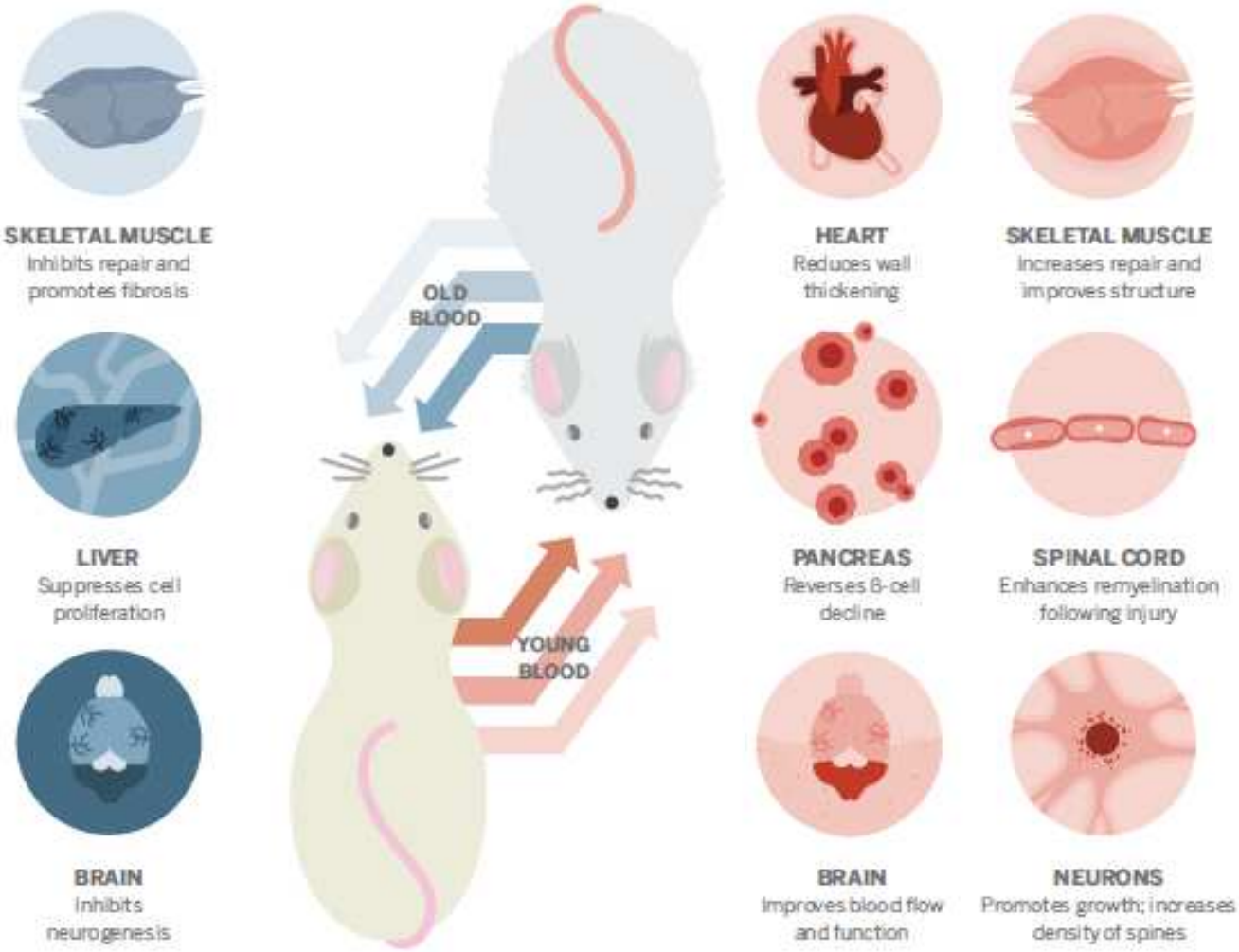
Margaret A. Goodell, and Thomas A. Rando Science 2015;350:1199-1204

Regenerace a buněčná terapie



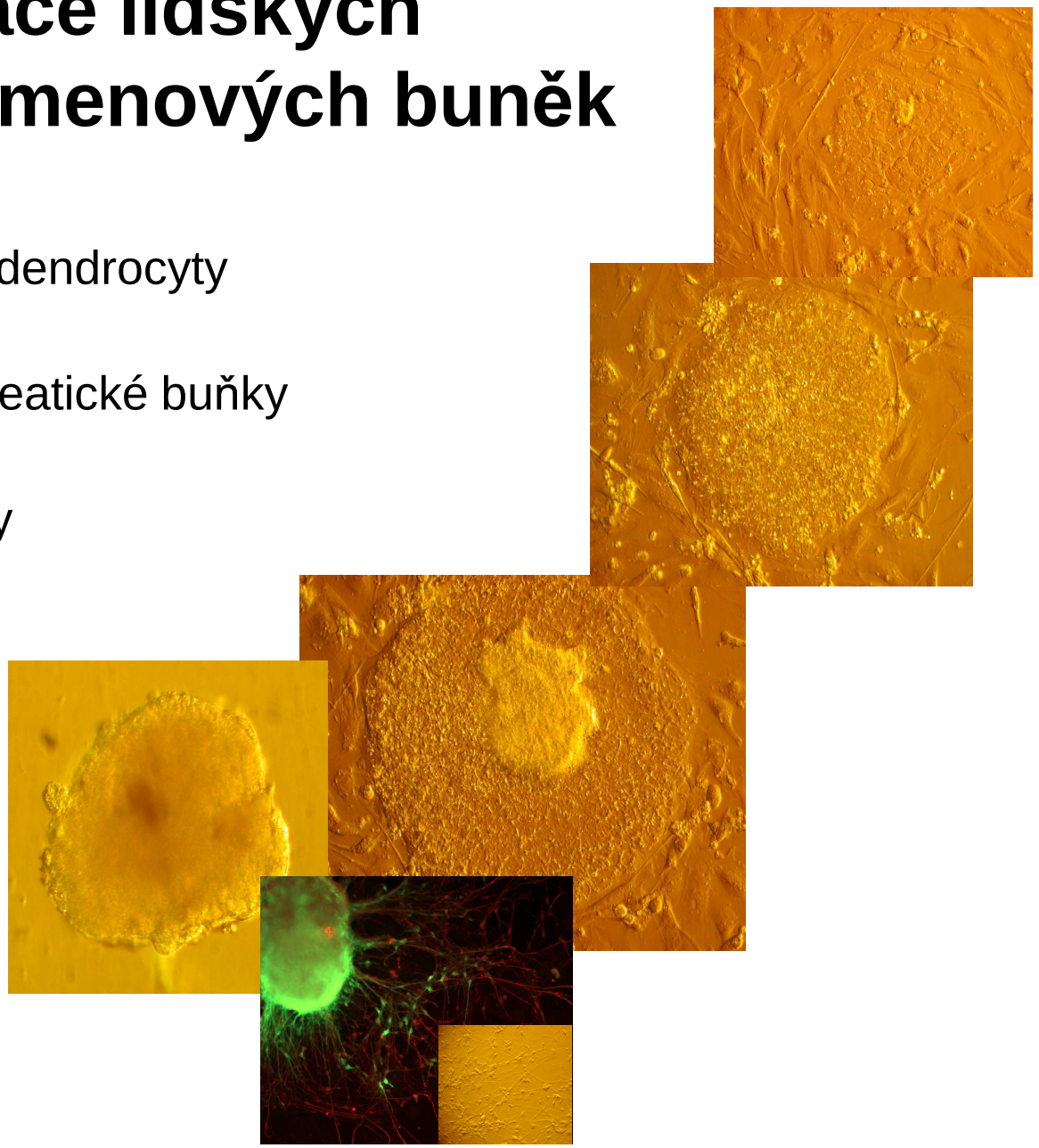
Young blood versus old blood

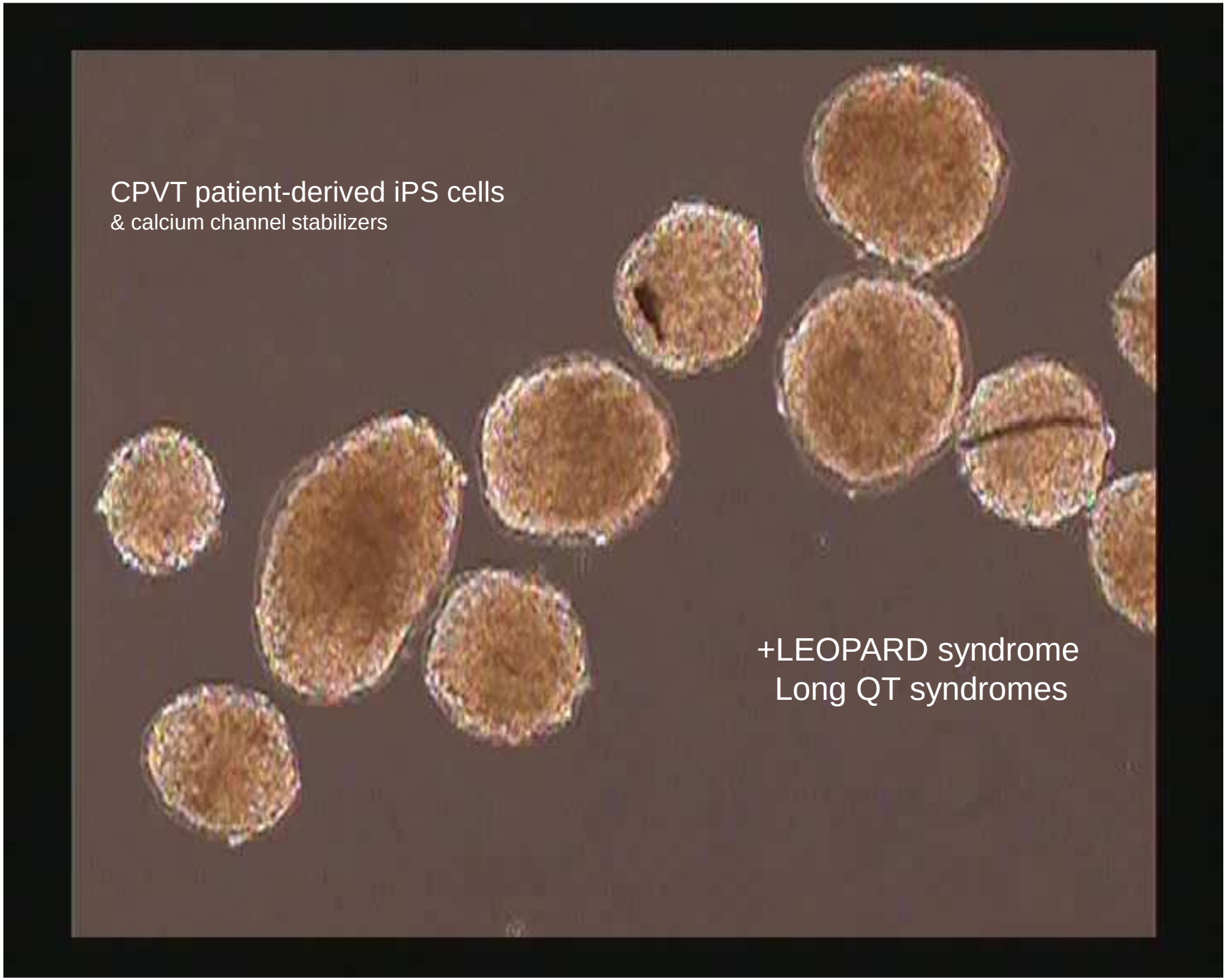
Factors in "young blood" activate stem cells and rejuvenate organs and cells in old mice. Factors in "old blood" appear to inhibit regenerative capacity in young mice.

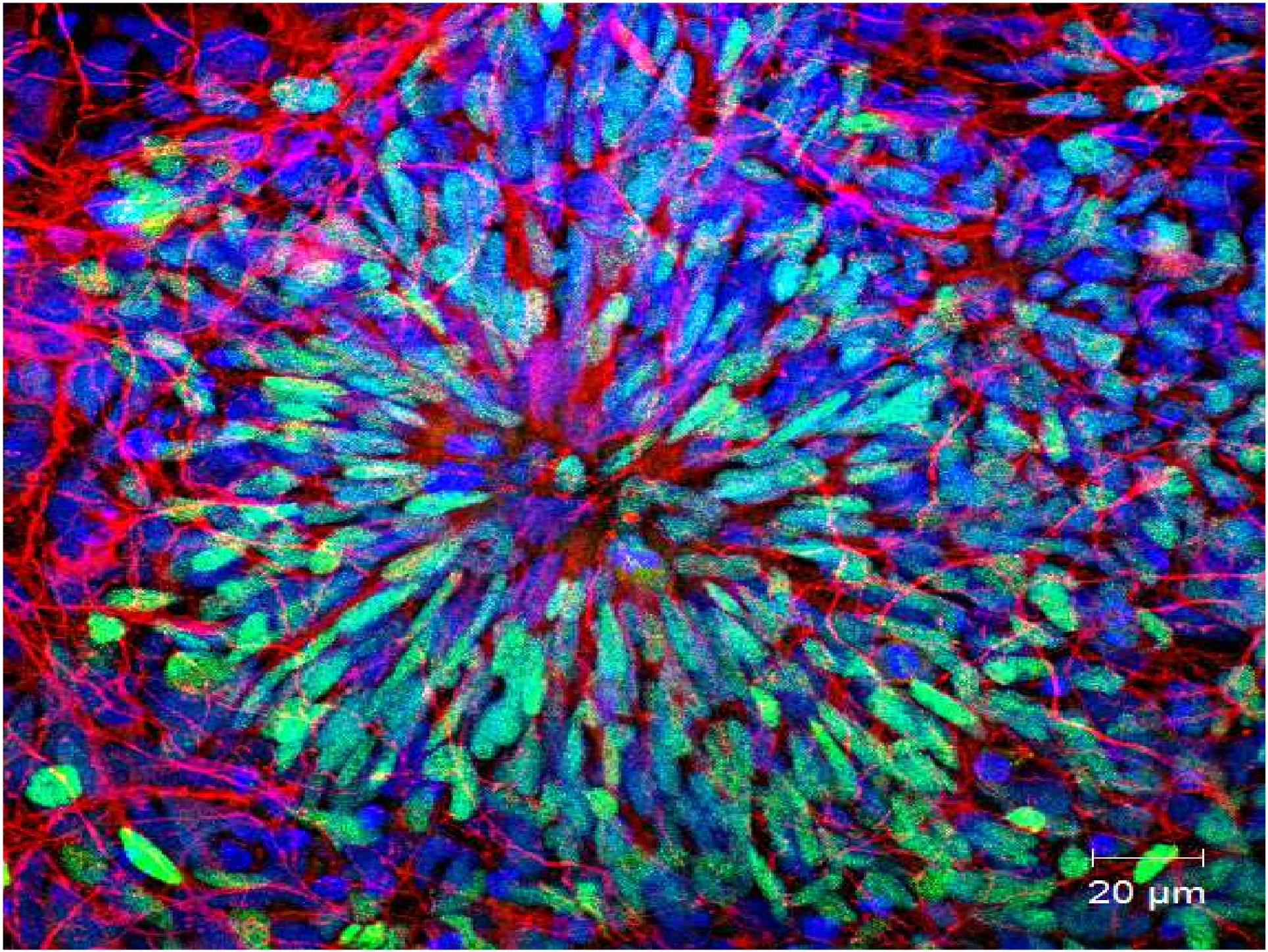


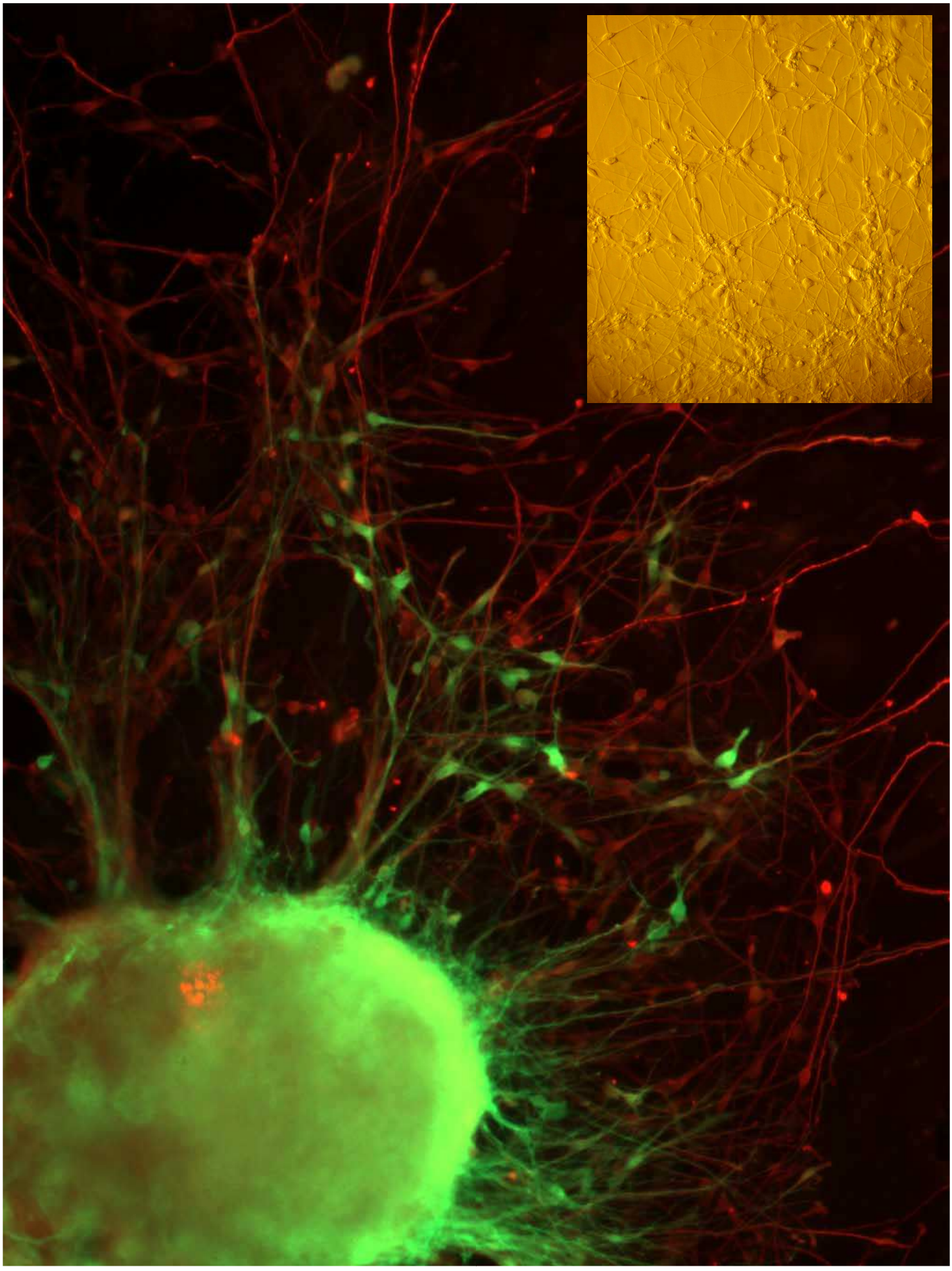
In vitro diferenciace lidských pluripotentních kmenových buněk

- Neurony, astrocyty, oligodendrocyty
- Kardiomyocyty
- Insulin-produkující pankreatické buňky
- Krevní buňky
- Imunokompetentní buňky
- Endoteliální buňky
- Buňky trofoblastu
- Respiratorní buňky
- Osteoblasty
- Hepatocyty
- Melanocyty
- Buňky prostaty
- Zárodečné buňky




CPVT patient-derived iPS cells
& calcium channel stabilizers
+LEOPARD syndrome
Long QT syndromes


20 µm

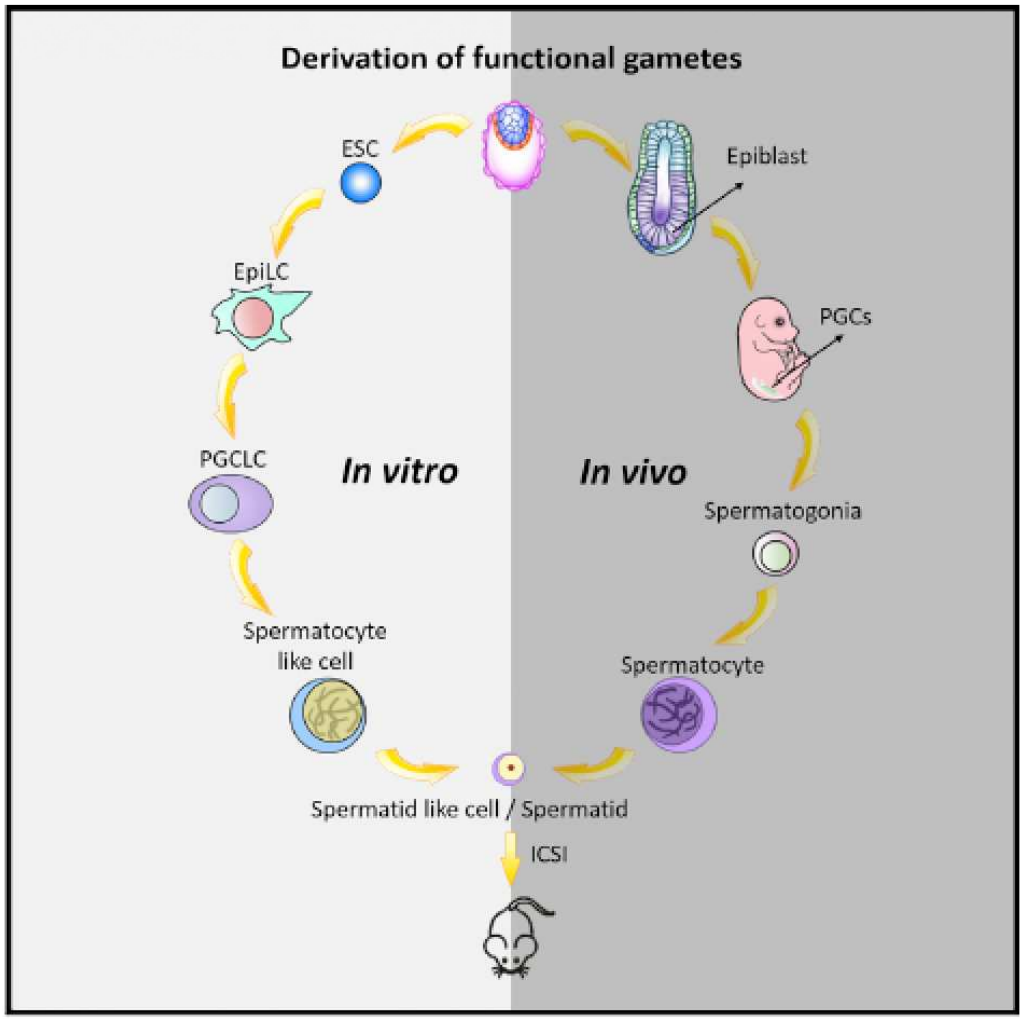


Article

Cell Stem Cell

Complete Meiosis from Embryonic Stem Cell-Derived Germ Cells In Vitro

Graphical Abstract



Authors

Quan Zhou, Mei Wang, Yan Yuan, ..., Xiao-Yang Zhao, Jiahao Sha, Qi Zhou

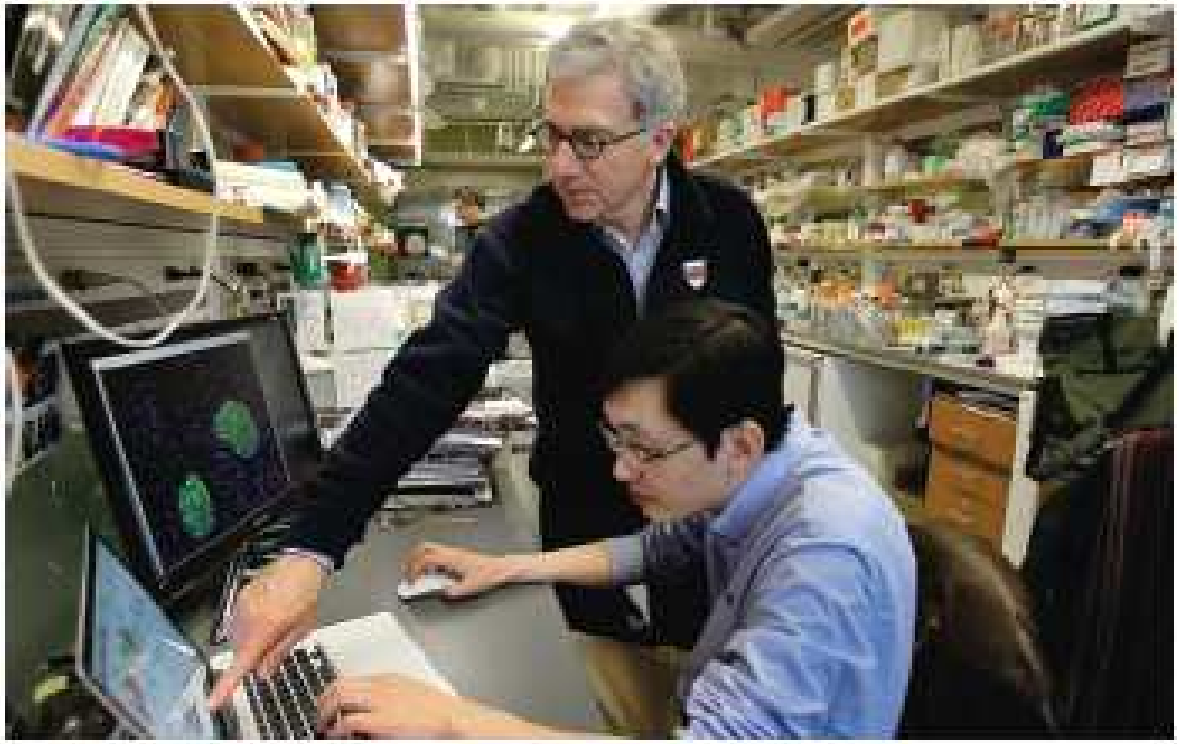
Correspondence

qzhou@ioz.ac.cn (Q.Z.),
shajh@njmu.edu.cn (J.S.),
zhaoxiaoyang@smu.edu.cn (X.-Y.Z.)

In Brief

In vitro production of haploid gametes could provide a treatment for infertility, but recapitulating meiosis in culture is a significant roadblock. Zhou et al. report the generation of haploid male gametes from mouse ESCs that can produce viable and fertile offspring, demonstrating functional reproduction of meiosis in vitro.

SUSANNE KUTTER BERKELEY/GETTY



A research team led by Douglas Melton (left) has made insulin-secreting cells using human stem cells.

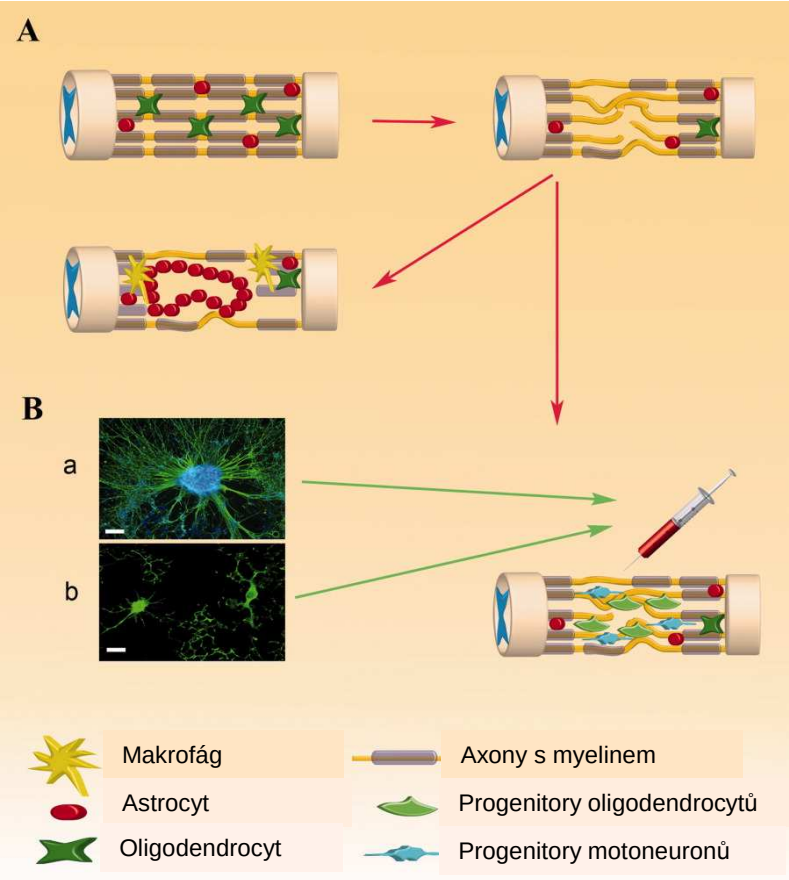
REGENERATIVE MEDICINE

Stem-cell success aids diabetes fight

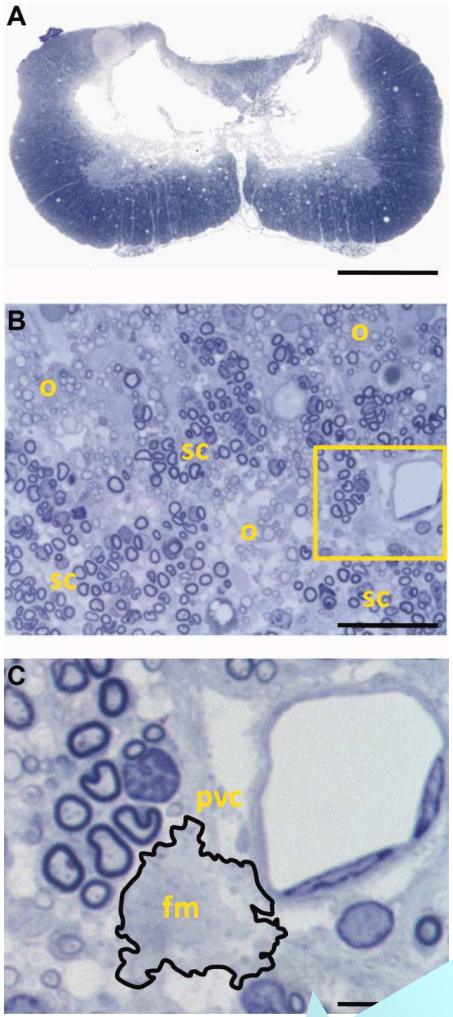
Now the challenge is to protect cell transplants from the immune systems of people with type 1 diabetes.

Příklad: traumatické poškození krční míchy, myelopatie a léčba kmenovými buňkami

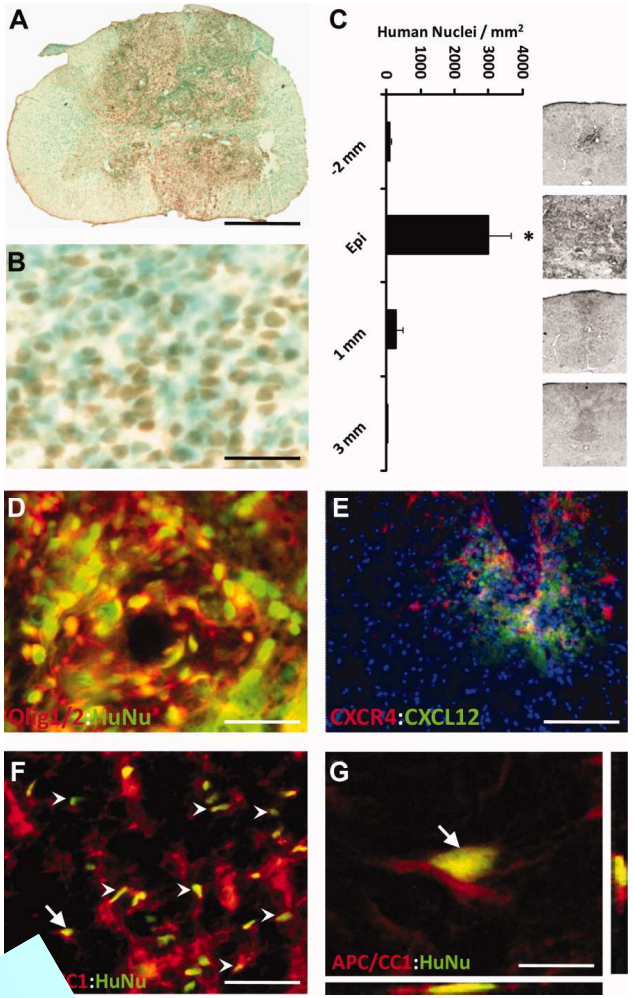
Patofyziologie a strategie léčby KB



Anatomie poškození



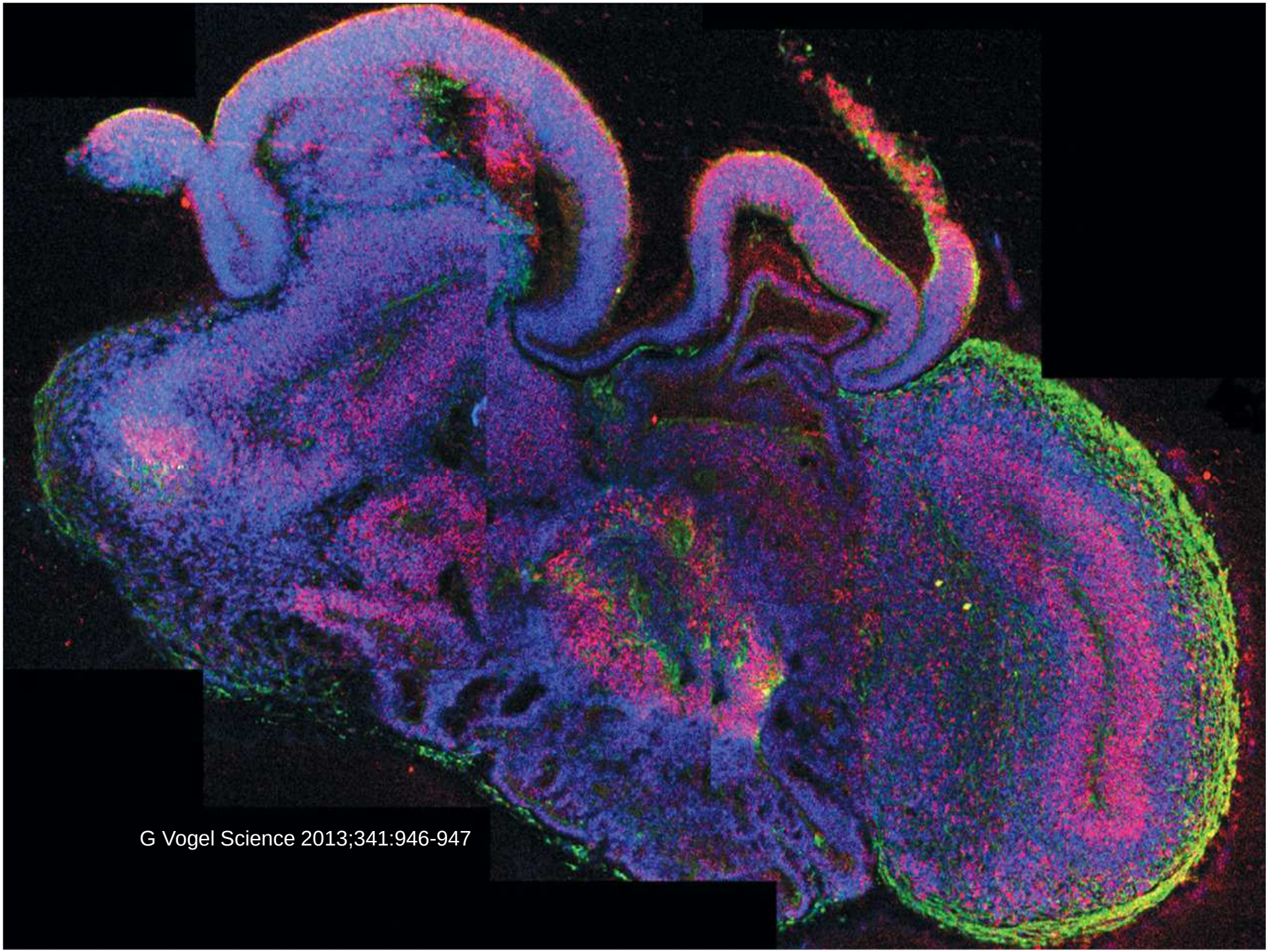
Stav po transplantaci



Výsledek: obnovení bílé a šedé hmoty v místě poškození
obnovení funkčnosti motorických neuronů
obnovení pohybových funkcí

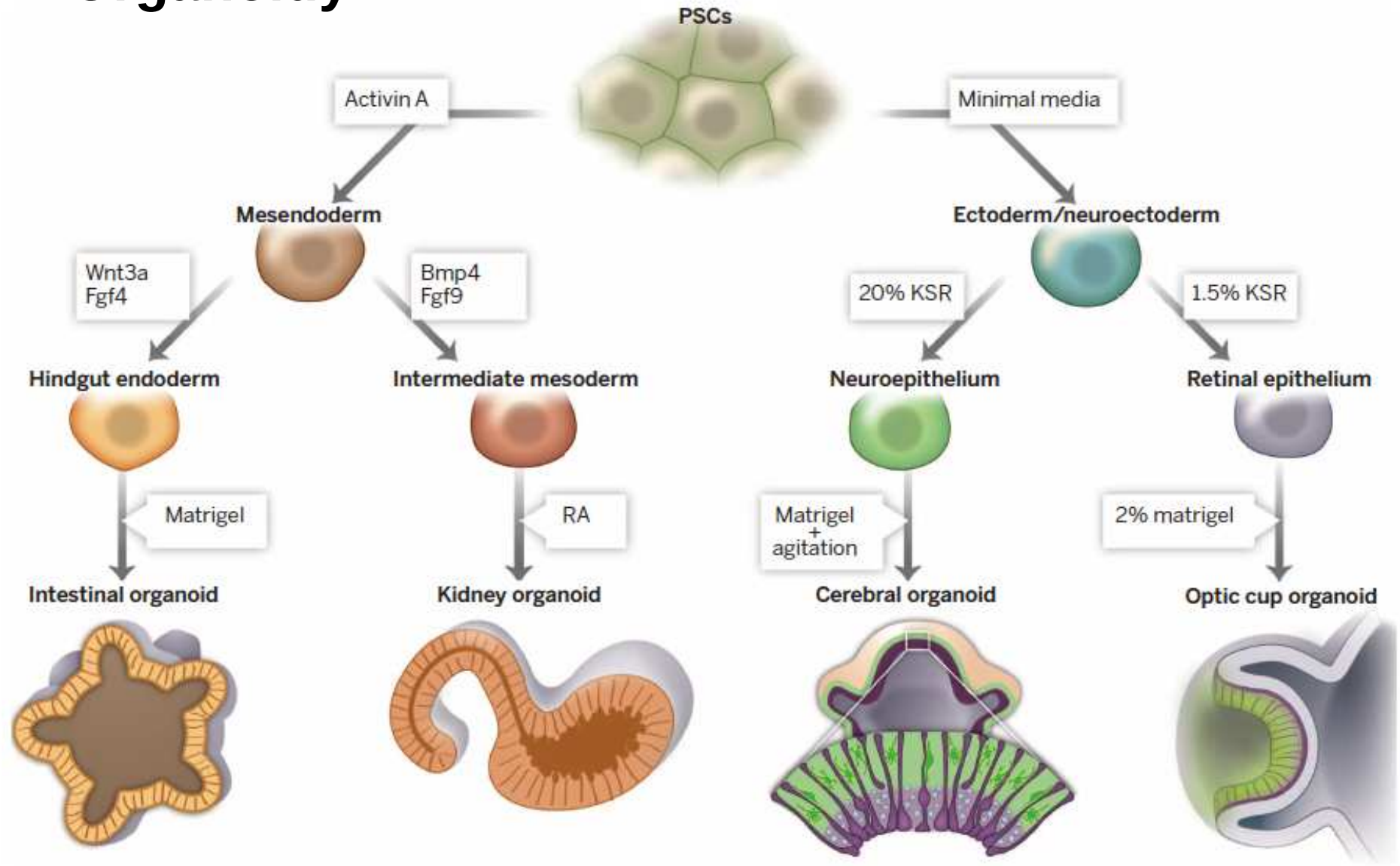
Model: laboratorní potkan

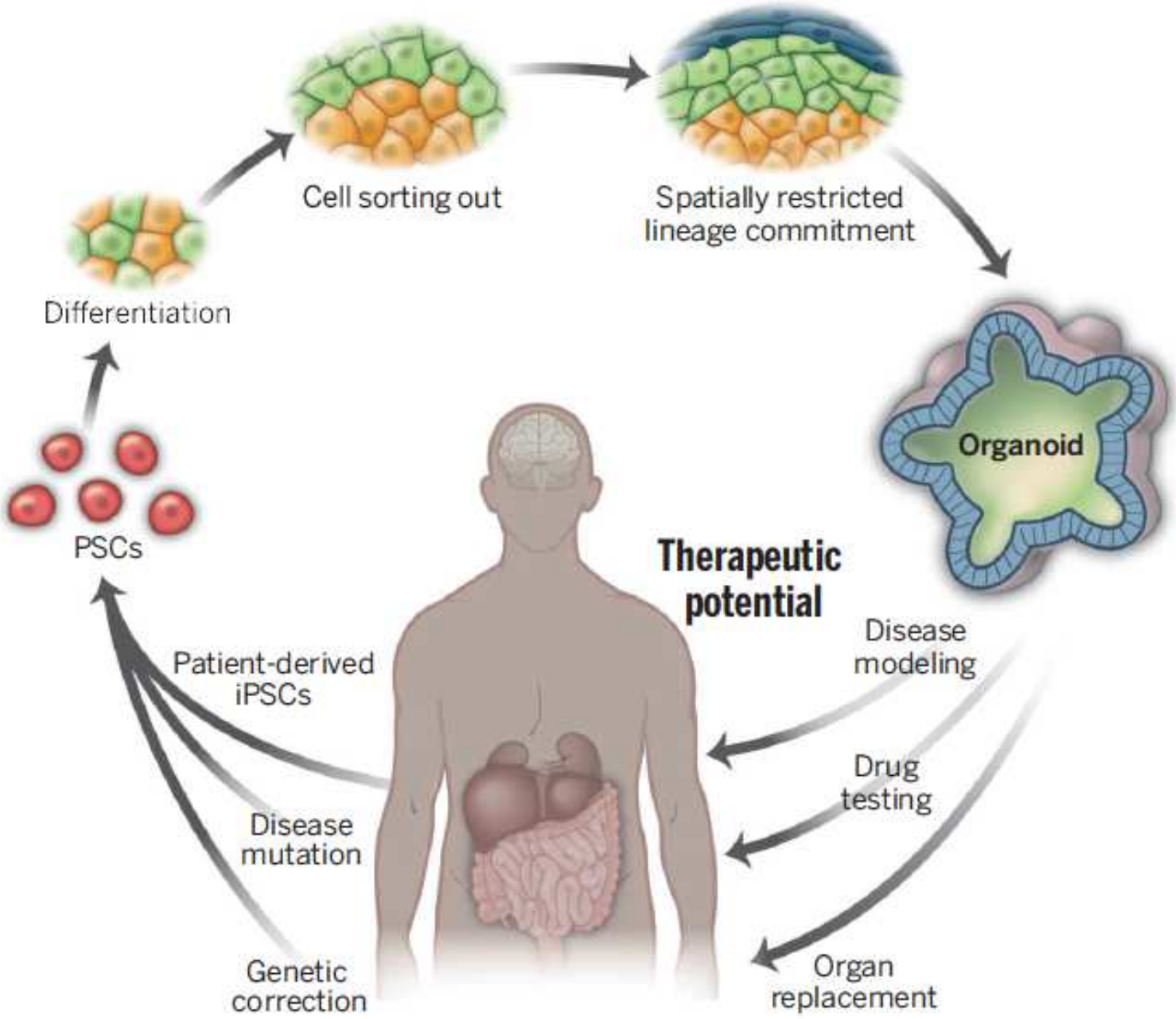
Adaptováno ze Stem Cells, 2010



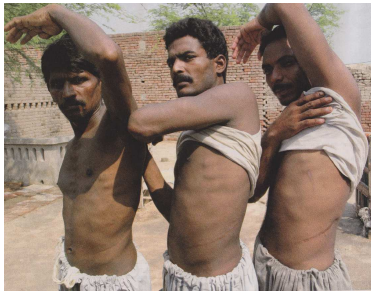
G Vogel Science 2013;341:946-947

Organoidy


PSCs
Activin A
Minimal media
Mesendoderm
Ectoderm/neuroectoderm
Wnt3a
Fgf4
Bmp4
Fgf9
20% KSR
1.5% KSR
Hindgut endoderm
Intermediate mesoderm
Neuroepithelium
Retinal epithelium
Matrigel
RA
Matrigel
+
agitation
2% matrigel
Intestinal organoid
Kidney organoid
Cerebral organoid
Optic cup organoid


Cell sorting out
Spatially restricted lineage commitment
Differentiation
Organoid
PSCs
Therapeutic potential
Patient-derived iPSCs
Disease modeling
Drug testing
Disease mutation
Genetic correction
Organ replacement

Mezidruhové chiméry, genetické manipulace a vývoj lidských orgánů ve zvířatech



Lidské orgány v praseti ???

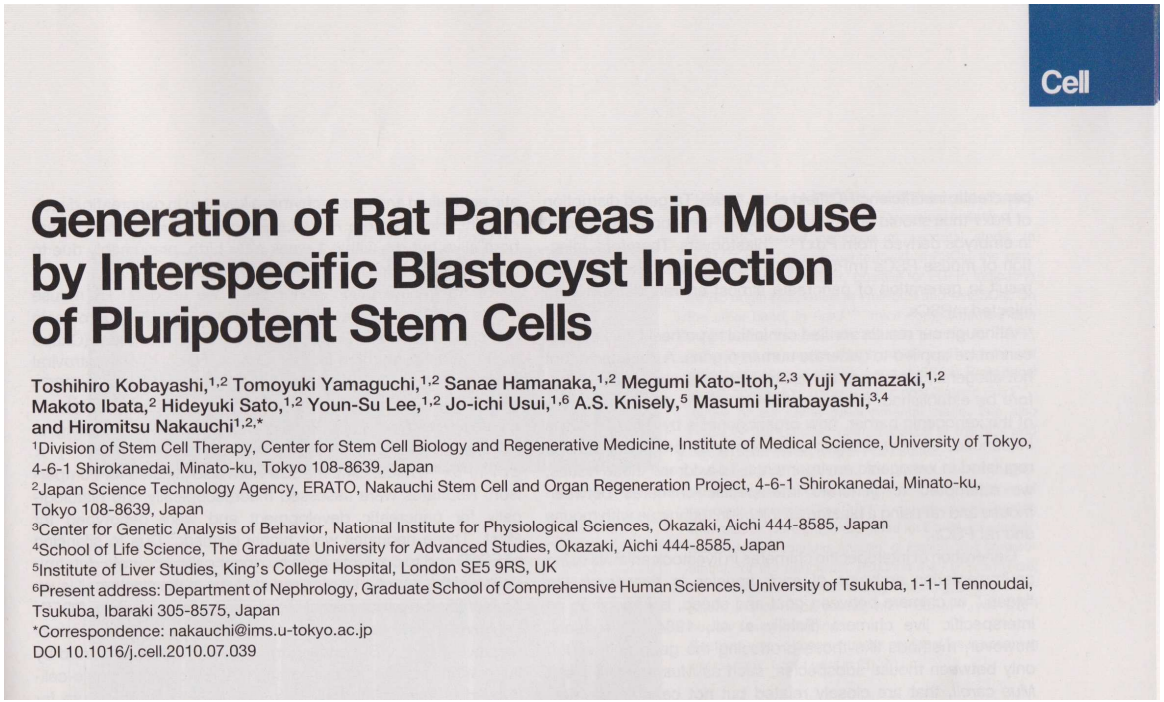
Krysí pankreas v myši

Normální vývoj jedince – mezidruhové chiméry

Vpravení normálních krysích kmenových buněk do myšího embrya - blastocysty

Časné myší embryo (blastocysta) s nefunkčním genem Pdx-1

Myš s nefunkčním genem Pdx-1
(Pdx-1 je klíčový gen pro vznik pankreatu)



Cell

Generation of Rat Pancreas in Mouse by Interspecific Blastocyst Injection of Pluripotent Stem Cells

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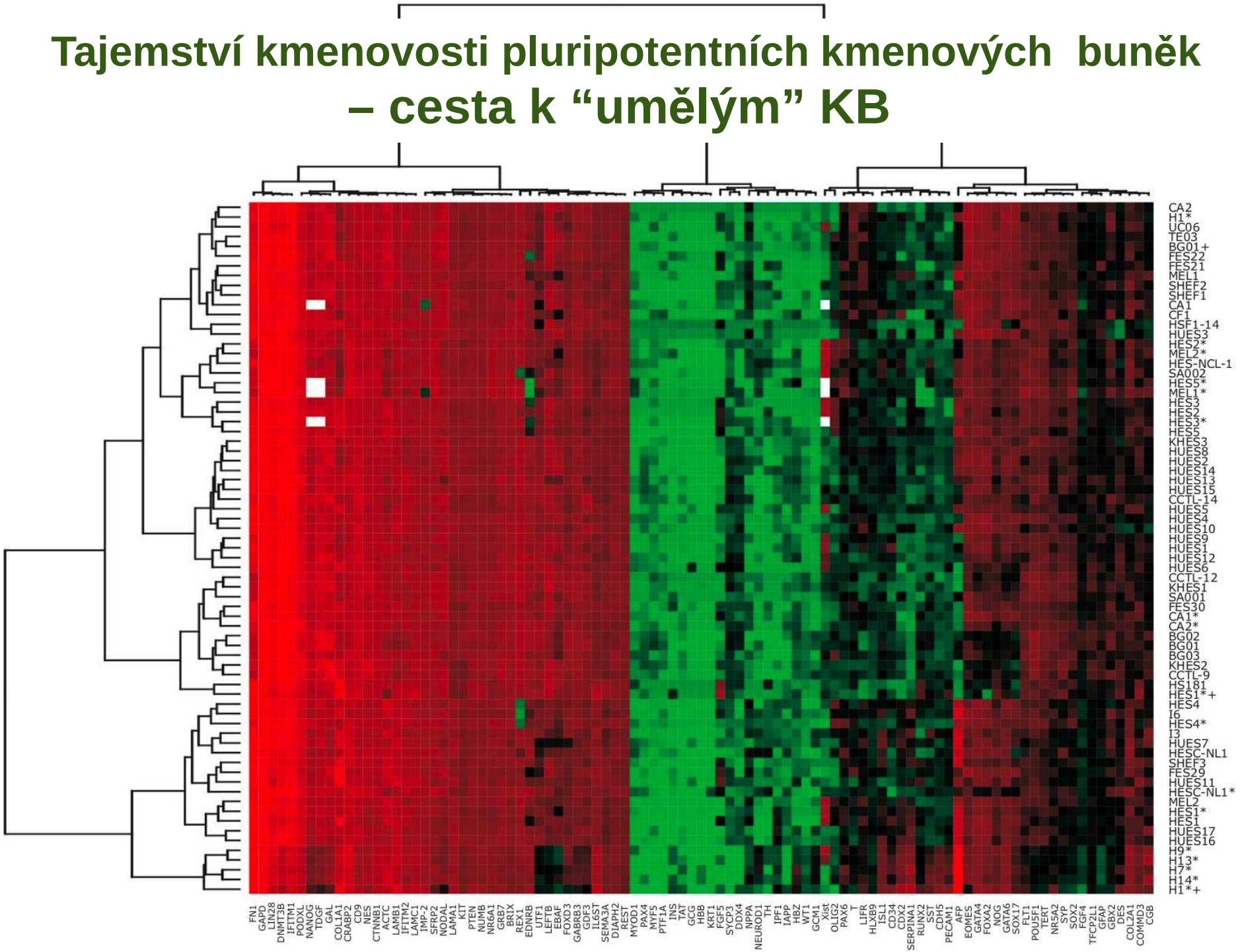
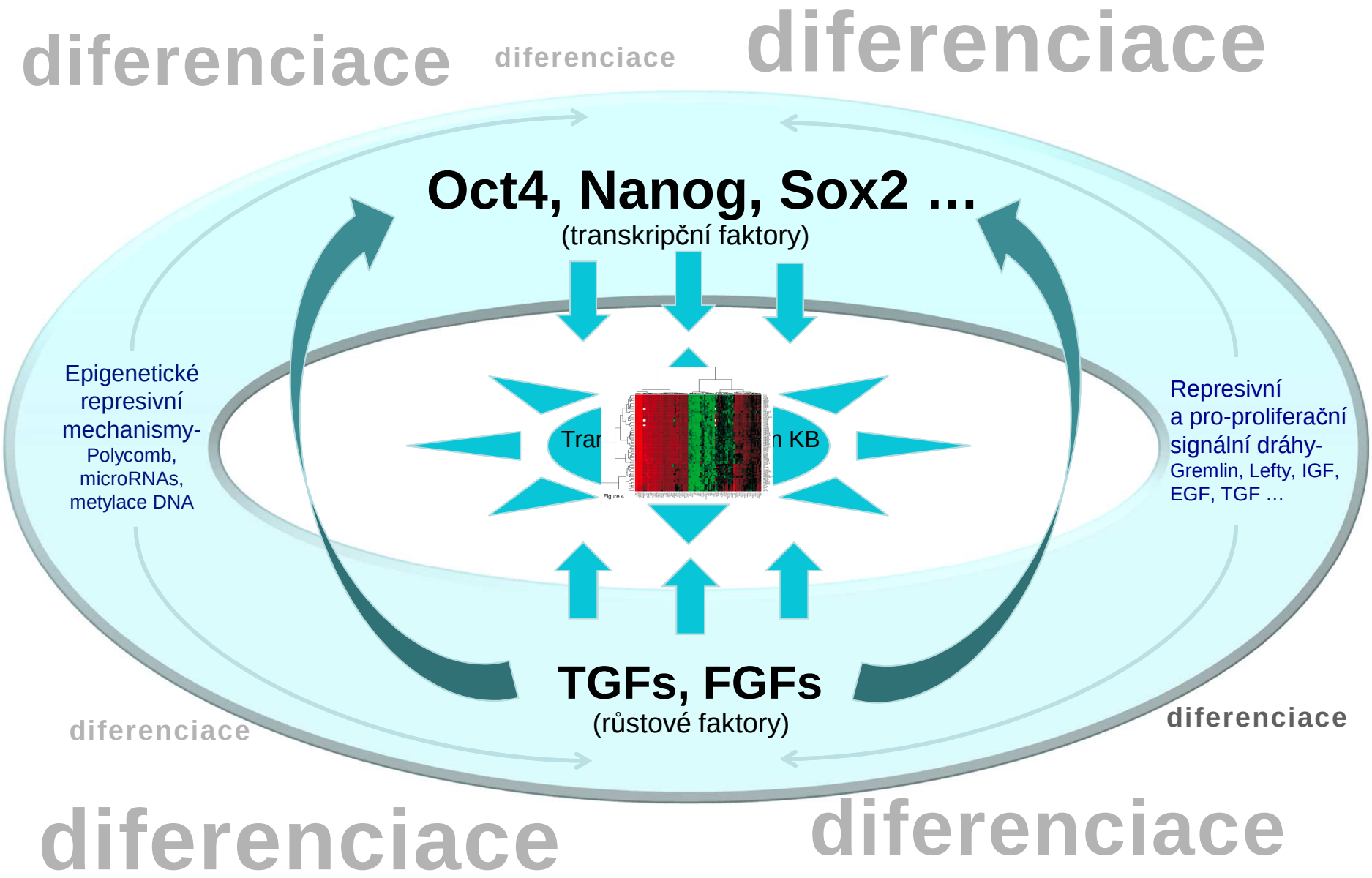
Tajemství kmenovosti pluripotentních kmenových buněk – cesta k “umělým” KB



Tajemství kmenovosti pluripotentních kmenových buněk



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

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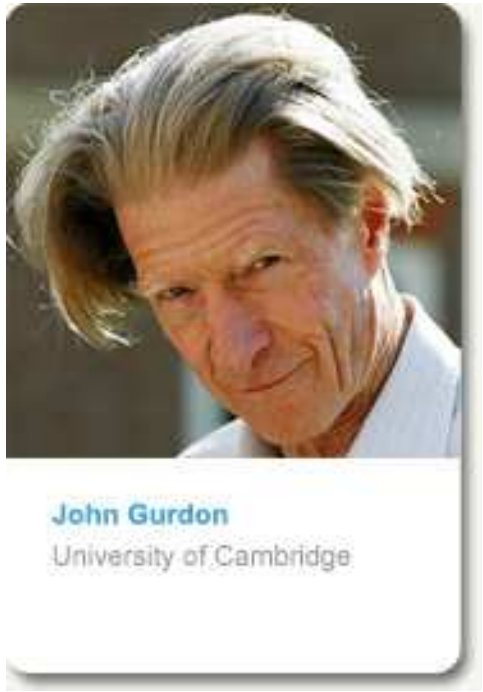
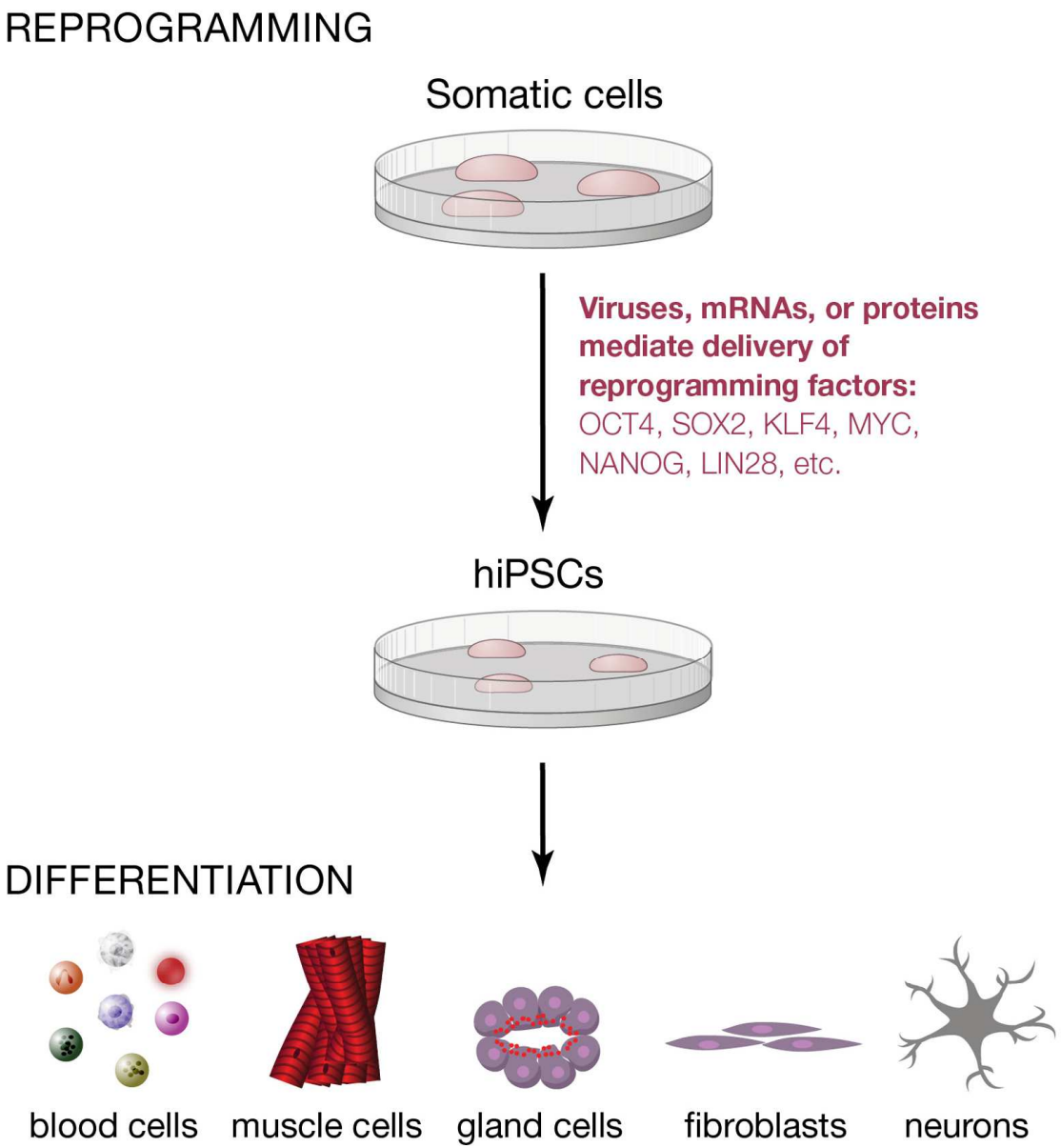
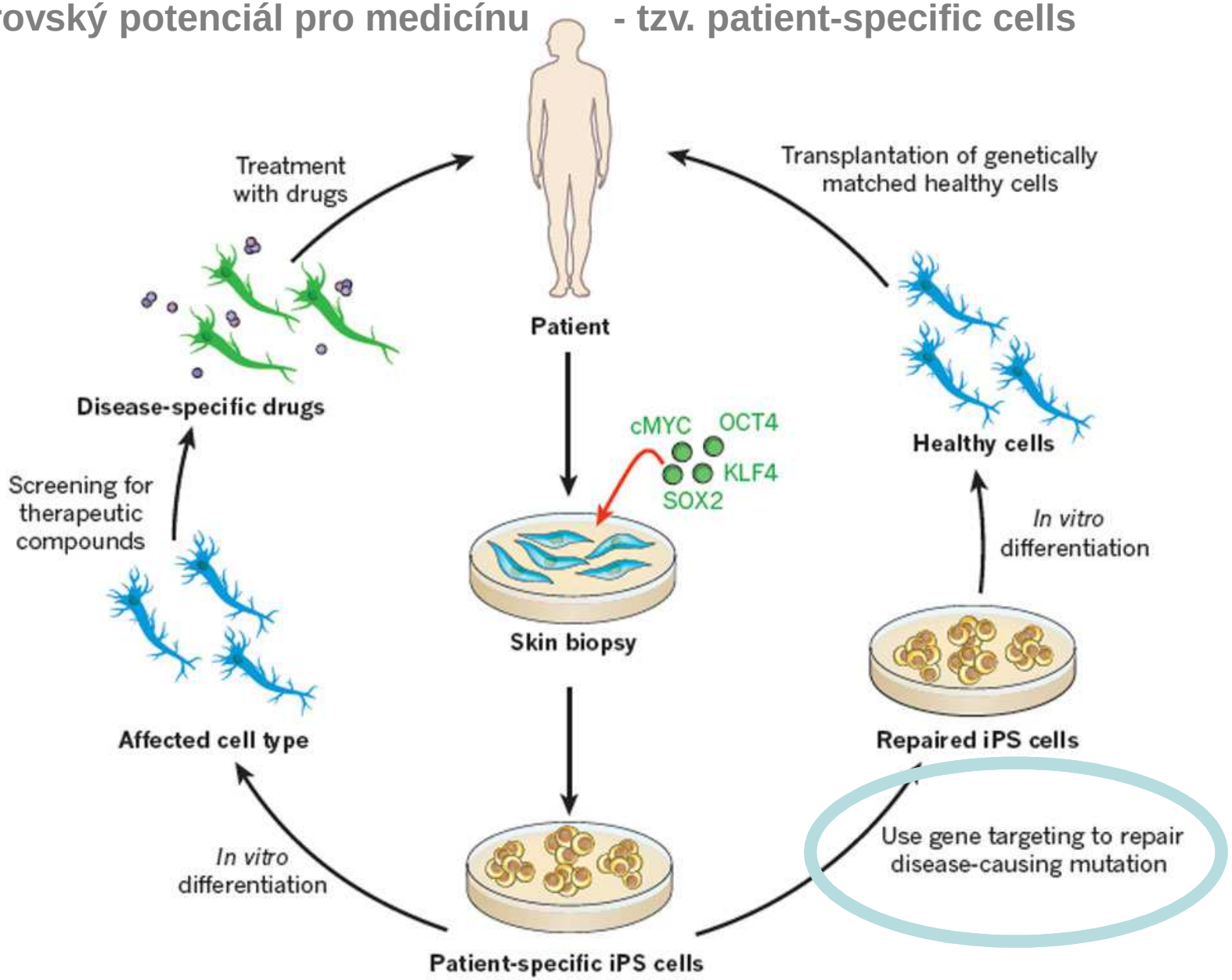
Albert Lasker basic medical research award 2009




REPROGRAMMING
Somatic cells
Viruses, mRNAs, or proteins mediate delivery of reprogramming factors:
OCT4, SOX2, KLF4, MYC, NANOG, LIN28, etc.
hiPSCs
DIFFERENTIATION
blood cells
muscle cells
gland cells
fibroblasts
neurons

Indukované pluripotentní kmenové buňky

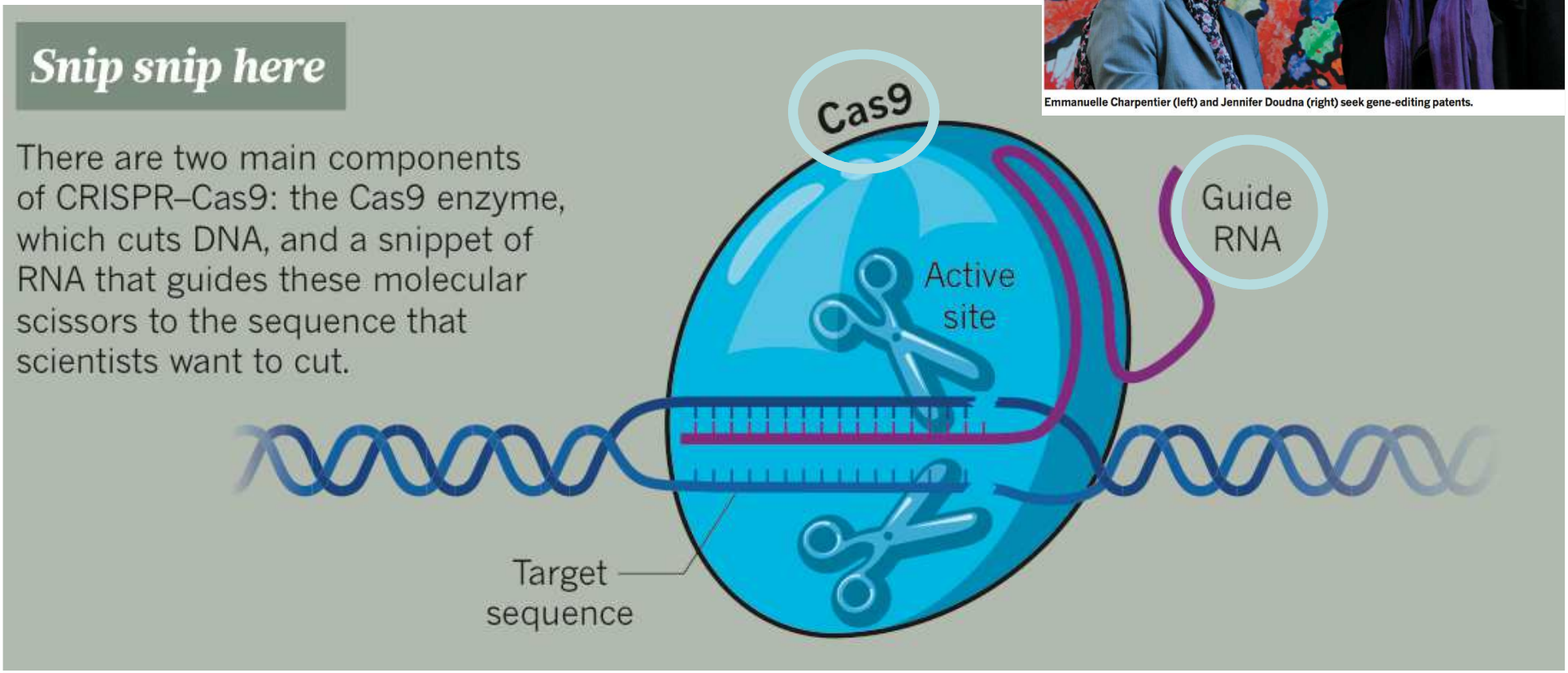
mají obrovský potenciál pro medicínu - tzv. patient-specific cells




CRISPR
EVERYWHERE
Illustration by Chris Labrooy ©nature

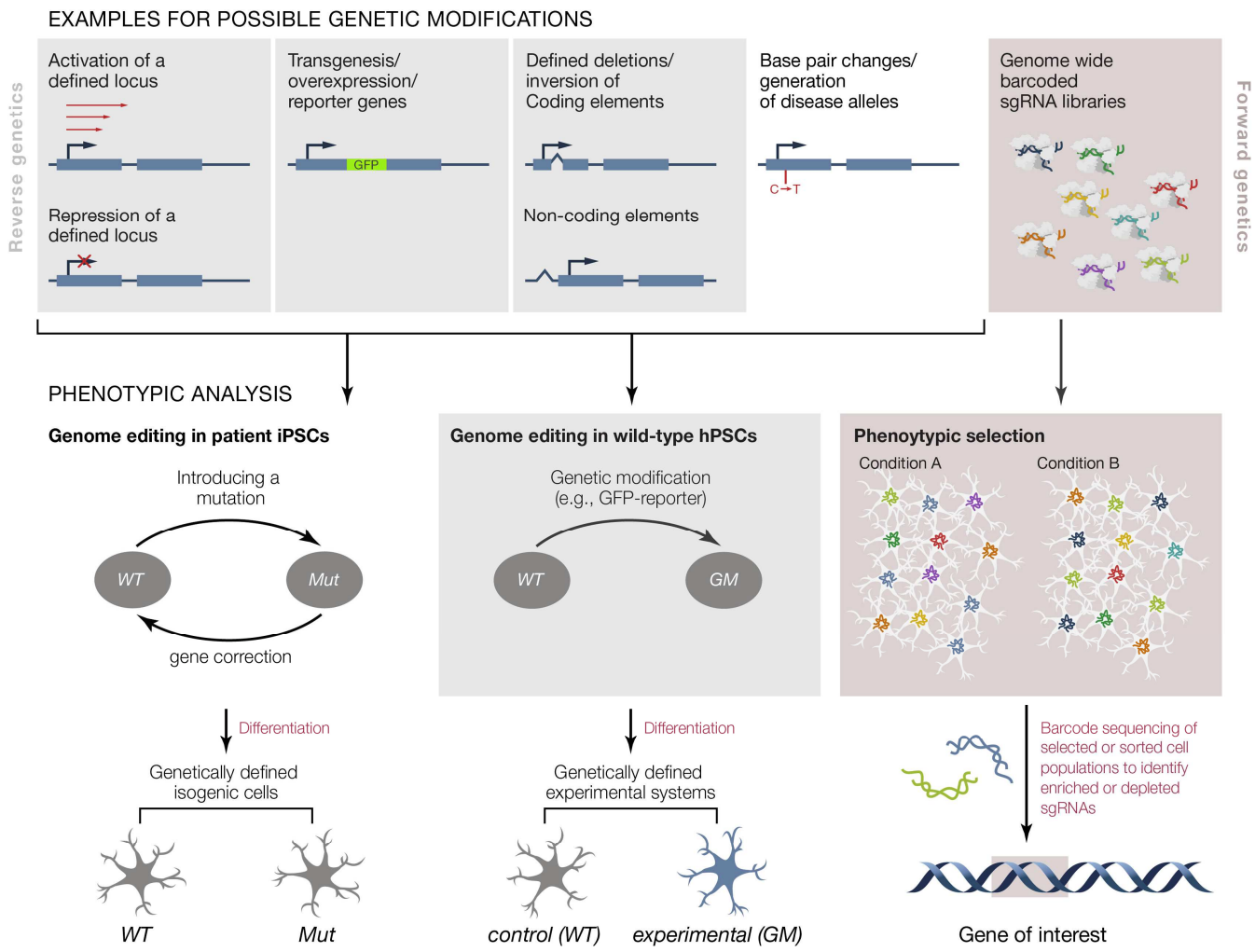
Další Nobelova cena?

Emmanuelle Charpentier (left) and Jennifer Doudna (right) seek gene-editing patents.



- **Editace genomu libovolných lidských buněk**
- **Editace genomu divokých zvířat a rostlin**

iPS cells meet CRISPR/Cas9 technology of genome editing



COMMENT

SHUTTERSTOCK

Don't edit the human germ line

Heritable human genetic modifications pose serious risks, and the therapeutic benefits are tenuous, warn **Edward Lanphier**, **Fyodor Urnov** and colleagues.

It is thought that studies involving the use of genome-editing tools to modify the DNA of human embryos will be published shortly[1].

There are grave concerns regarding the ethical and safety implications of this research. There is also fear of the negative impact it could have on important work involving the use of genome-editing techniques in somatic (non-reproductive) cells.

We are all involved in this latter area of work. One of us (F.U.) helped to develop the first genome-editing technology, zinc-finger nucleases[2] (ZFNs), and is now senior scientist at the company developing them, Sangamo BioSciences of Richmond, California. The Alliance for Regenerative Medicine (ARM; in which E.L., M.W. and S.E.H. are involved), is an international organization that represents more than 200 life-sciences companies, research institutions, non-profit organizations, patient-advocacy groups and investors focused on developing and commercializing therapeutics, including those involving genome editing.

Genome-editing technologies may offer a powerful approach to treat many human diseases, including HIV/AIDS, haemophilia, sickle-cell anaemia and several forms of cancer[3]. All techniques currently in various stages of clinical development focus on modifying the genetic material of somatic cells, such as T cells (a type of white blood cell). These are not designed to affect sperm or eggs.

In our view, genome editing in human embryos using current technologies could have unpredictable effects on future generations. This makes it dangerous and ethically unacceptable. Such research could be exploited for non-therapeutic modifications. We are concerned that a public outcry about such an ethical breach could hinder a promising area of therapeutic development, namely making genetic changes that cannot be inherited.

At this early stage, scientists should agree not to modify the DNA of human reproductive cells. Should a truly compelling case ever arise for the therapeutic benefit of germline modification, we encourage an open discussion around the appropriate course of action.

EDITING TOOLS

Genome editing of human somatic cells aims to repair or eliminate a mutation that could cause disease. The premise is that corrective changes to a sufficient number of cells carrying the mutation — in which the genetic fixes would last the lifetimes of the modified cells and their progeny — could provide a 'one and done' curative treatment for patients.

For instance, ZFNs are DNA-binding proteins that can be engineered to induce a double-strand break in a section of DNA. Such molecular scissors enable researchers to 'knock out' specific genes, repair a mutation or incorporate a new stretch of DNA into a selected location.

Sangamo BioSciences is conducting clinical trials to evaluate an application of genome editing as a potential 'functional cure' for HIV/AIDS[4]. The hope is that

Bioetická komise je v souladu se světovou odbornou veřejností přesvědčena, že jakékoliv úvahy o editaci genomu gamet v humánní klinické praxi vyvolávají závažné etické a sociální pochybnosti, přinášejí nepředvídatelná rizika a jsou předčasné.

Bioetická komise, v souladu s Úmluvou o lidských právech a biomedicíně Rady Evropy, kterou Česká republika ratifikovala, **žádá vědeckou obec neprovádět výzkum, který se týká editace genomu lidských gamet** a žádá poskytovatele finančních prostředků v České republice, aby nepodporovali takové projekty.

Současně Bioetická komise podporuje názor světové odborné veřejnosti, že **s výjimkou lidských gamet je důležité výzkum zahrnující editaci genomu podporovat a žádným způsobem nechce brzdit další vývoj a debatu v této oblasti výzkumu**.