7. Doppelstunde am 18.11.2015

Rückblick

Arten von Stammzellen und ihre Anwendung in Grundlagenforschung und Medizin

Stammzellarten und ihr Differenzierungspotenzial

Ausgewählte Beispiel der Anwendung von Stammzellen in der Forschung und in Therapieversuchen

ESCs m←→h ..... Embryoid bodies, transgenic mice
PGCs

~ 300 hESC lines at the NIH
2000 hiPSC lines wordlwide

SSCs: Brain
skin
Gut
heart

NEW iPSC Strategy:
Duanqing Pei: C-Jun inhibts iPSC generation
Y27632 (ROCK-I) better than RepSox

Instead of OKSM: Id1, Sall4, Lrh1, Jhdm1b, Glis1 and Jdp2 which inhibits cJun

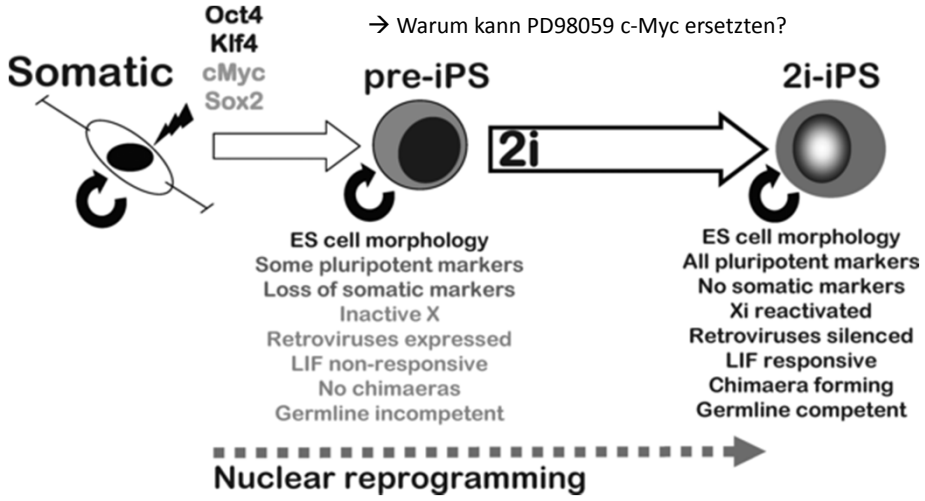
Artificial SCs (aSCs):

iPSCs (retroviral transfection with Oct4, Sox2, Klf4 und c-Myc)
SCNT-ESCs (ntESCs) → reproduktived ← → therapeutisches Klonen
si-ESCs (stress induced)
CSCs (cancer)

Polygenetisch bedingte Krankheitsmodelle
e.g. Glia-associated neuropsychiatric dissorders

Mitochondrial replacement therapy (MRT)

Figure 6. Transfection-Induced Reprogramming Commonly Stalls at a Pre-Pluripotent Stage and Can Be Advanced to Completion by Mek/Erk Inhibition



→ Warum kann PD98059 c-Myc ersetzten?

Silva J, Barrandon O, Nichols J, Kawaguchi J, Theunissen TW, et al. (2008) Promotion of Reprogramming to Ground State Pluripotency by Signal Inhibition. PLoS Biol 6(10): e253. doi:10.1371/journal.pbio.0060253
http://127.0.0.1:8081/plosbiology/article?id=info:doi/10.1371/journal.pbio.0060253

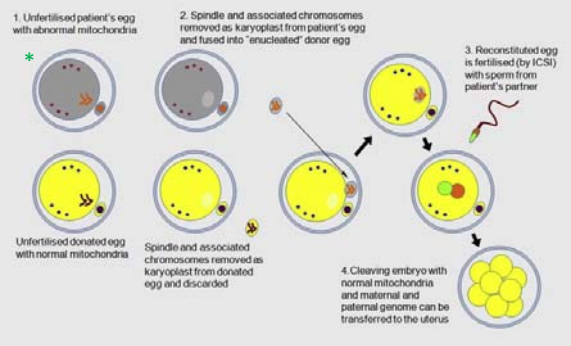
PLOS | BIOLOGY

Figure 6. Transfection-Induced Reprogramming Commonly Stalls at a Pre-Pluripotent Stage and Can Be Advanced to Completion by Mek/Erk Inhibition

Transduction of somatic cells with *Oct4*, *Klf4*, *c-Myc*, and *Sox2* transgenes gives rise at early stages to cells that have undifferentiated morphology and express some markers of ES cells. These first appearing ES cell–like cells do not convert to full pluripotency, even if continuously propagated. In the case of NS cells, partially reprogrammed cells are evident just 3 d after transduction The block to full pluripotency can be released by applying small molecule inhibitors (2i) of the Mek/Erk pathway and of glycogen synthase kinase-3 in the presence of LIF. Transition to authentic iPS cell status occurs rapidly and at high efficiency. Alternatively, 2i/LIF may be applied shortly after transduction before emergence of pre-iPS cell colonies. In this case fully reprogrammed iPS cells are isolated directly, reflecting rapid transit through, or possibly bypass of, the pre-iPS cell stage. In either scenario use of 2i enables isolation of authentic iPS cells without recourse to reporter genes or unreliable morphological assessment. In addition, reprogramming of neural stem cells to ground state pluripotency in the presence of 2i/LIF is not enhanced by exogenous *Sox2* and may proceed with only 1–2 integrations of *Oct4*, *Klf4*, and, optionally, *c-Myc*. Although, germline competent iPS cells can be obtained in standard ES cell culture conditions without use of 2i. This occurs at low frequency over 3 wk or longer. We surmise that this low-efficiency ''stochastic'' pathway avoids passing through or becoming trapped in the pre-iPS cell stage.

doi:10.1371/journal.pbio.0060253.g006

SCNT = Somatic Cell Nuclear Transfer
Spindeltransfer = Transfer of a karyoblast from an foreign egg



Spindle transfer technique.

* Beim SCNT wird statt dem Ei eine somatische Zelle genommen.

Problem:
Heteroplasmie

Three-parent in vitro fertilization: gene replacement for the prevention of inherited mitochondrial diseases
Paula Amato , Masahito Tachibana , Michelle Sparman , Shoukhrat Mitalipov
Fertility and Sterility, Volume 101, Issue 1, 2014, 31 – 35. http://dx.doi.org/10.1016/j.fertnstert.2013.11.030

SCNT = Somatic Cell Nuclear Transfer
Spindeltransfer = Transfer of a karyoblast from an foreign egg

Umbenannt in :
Mitochondrial replacement therapy (MRT) = Reproduktives Klonen von Menschen

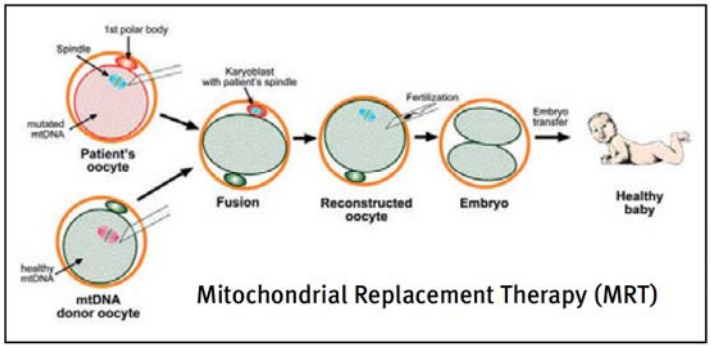


Illustration: OHSU

Polygenetisch bedingte Krankheitsmodelle
e.g. Glia-associated neuropsycuiatric disorders

A Competitive Advantage by Neonatally Engrafted Human Glial Progenitors Yields Mice Whose Brains Are Chimeric for Human Glia

Martha S. Windrem,[1] Steven J. Schanz,[1] Carolyn Morrow,[1] Jared Munir,[1] Devin Chandler-Militello,[1] Su Wang,[1] and Steven A. Goldman[1,2]

J Neurosci. 2014 Nov 26; 34(48): 16153–16161. doi: 10.1523/JNEUROSCI.1510-14.2014PMCID: PMC4244478

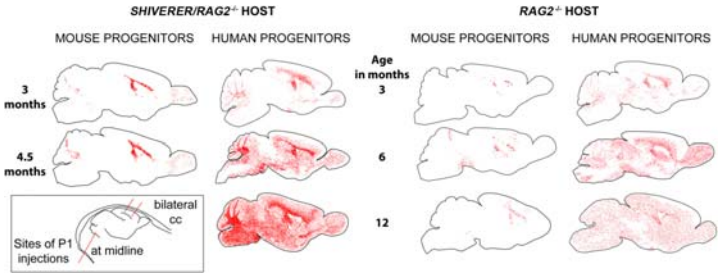
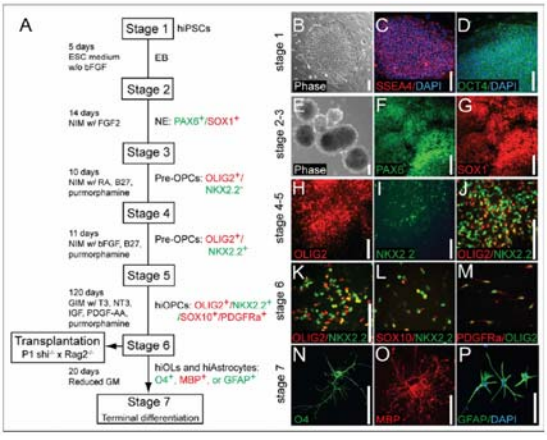


Figure 1. hGPCs colonize both wild-type and myelin-deficient immunodeficient host brain. hGPCs neonatally transplanted into either congenitally hypomyelinated *shivererrag2/* mice (left columns) or normally myelinated *rag2/*mice (right columns) disperse and expand broadly throughout the brain as a function of age, and do so more aggressively than allografted mouse GPCs. hGPCs reach higher density in white matter than gray matter of the hypomyelinated *shiverer*, in contrast to their relatively uniform distribution in normally myelinated brain (right). Same-species neonatal allografts of EGFP-expressing mouse GPCs migrate and expand substantially less. Red dots indicate individual donor GPCs, as labeled byhumannuclear antigen (human GPCs) or anti-GFP (mouse GPCs). Cells were mapped in 20m sections using Stereo Investigator. Inset, Bottom left, Sites of neonatal injection, given anteriorly and posteriorly into the corpus callosum bilaterally, and as a single injection into the cerebellar peduncle.

Human iPSC-derived oligodendrocyte progenitors can myelinate and rescue a mouse model of congenital hypomyelination

Su Wang,[1] Janna Bates,[1] Xiaojie Li,[1] Steven Schanz,[1] Devin Chandler-Militello,[1] Corri Levine,[1] Nimet Maherali,[2] Lorenz Studer,[3] Konrad Hochedlinger,[2] Martha Windrem,[1] and Steven A. Goldman[1,*]Cell Stem Cell. 2013 Feb 7; 12(2): 252–264. doi: 10.1016/j.stem.2012.12.002



160 Tage !

Figure 1. hiPSCs can be directed into OPC fate
(A) A schematic protocol for directed differentiation of hiPSC into OPCs. Embryoid bodies (EBs) were differentiated from undifferentiated hiPSC (Stage 1) from DIV 0-5. EBs were then differentiated as neuroepithelial (NE) cells in neural induction media (NIM; see Methods) with bFGF. **(B-D)** Undifferentiated hiPSCs (stage 1) and hiPSC colonies expressed the pluripotency markers SSEA-4 and OCT4. EBs **(E)** and neuroepithelial cells **(F-G)** could be generated from hiPSC (stages 2-3). hiPSC-derived EBs at this stage expressed the neuroepithelial markers PAX6 and SOX1. **(H-I)** OLIG2+/NKX2.2- early GPCs appeared under the influence of RA and purmorphamine, a small molecule agonist of sonic hedgehog signaling. By stage 4, OLIG2 was expressed in early pre-OPCs, which then serially developed NKX2.2 expression. **(J)** OLIO2+/NKX2.2- early pre-OPCs were differentiated into later-stage OLIG2+/NKX2.2+ pre-OPCs, when RA was replaced by bFGF at stage 5. **(K-M)** Pre-OPCs were further differentiated into bipotential OPCs in glial induction media (GIM; see Methods), supplemented with PDGF AA, T3, NT3 and IGF. Stage 6 was extended as long as 3- 4 months, so as to maximize the production of myelinogenic OPCs. By the time of transplant, these cells expressed not only OLIG2 and NKX2.2 **(K)**, but also SOX10 **(L-M)** and PDGFRα **(M)**. By the end of stage 6, hiPSC OPCs could be identified as OLIG2+/NKX2.2+/SOX10+/PDGFRα+. **(N-P)** In vitro terminal differentiation of hiPSC OPCs into human iPSC-derived oligodendrocytes (hiOLs), identified by O4+ **(N)** and MBP+ **(O)**. Oligodendrocytes and GFAP+ astrocytes **(P)** arose with reduction in glial mitogens. Scale: **B-N, P**, 100 μm; **O**, 25 μm.

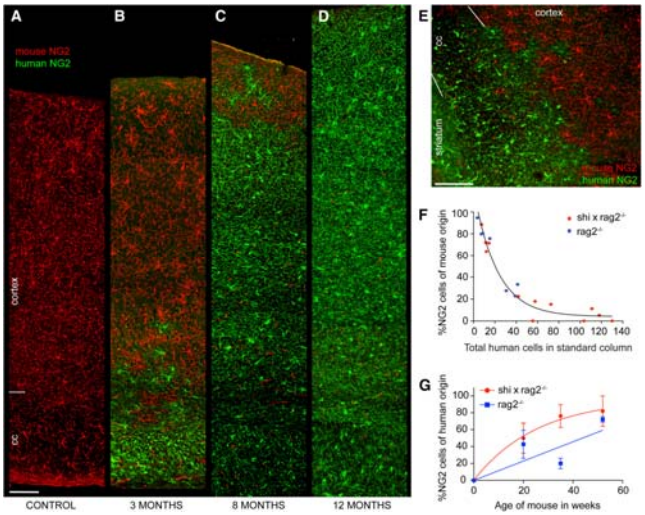


Figure 3. Progressive domination of murine forebrain by human glial progenitors. hGPC replacement of mouse glial progenitor cells (mGPCs) in both *shiverer* and normally myelinated *rag2*/ mice, visualized using species-specific antibodies to the GPC chondroitin sulfate proteoglycan NG2. ***A***, An unengrafted control showing the normal distribution of mouse NG2cells (red), spanning the distance from the ventricular ependyma to the pial surface; 3 months of age. ***B–D***, Analogous radial strips spanning the ventricular to the pial surface, including the corpus callosum and cortex, of *shivererrag2*/ mice engrafted neonatally with hGPCs. These images, taken at 3 (***B***), 8 (***C***), and 12 (***D***) months of age, show the systematic expansion of hNG2-defined hGPCs from the callosum to the cortical mantle, and the concurrent displacement of endogenous murine NG2cells. Red represents mouse NG2; green represents human NG2. ***E***, Higher-magnification image of the mGPC-hGPC border in a 3-month-old myelin wild-type *rag2*/ mouse demonstrates the sharp demarcation between the advancing human and retreating mouse glial progenitors. In myelin wild-type mice, by this time-point, hGPCs have replaced mGPCs throughout the corpus callosum, the hippocampus, and lower layers of cortex; mouse NG2cells still dominate the superficial layers of the cortex. ***F***, The replacement of host mouse cells by transplanted hGPCs in both *shiverer/rag2*/(red dots) and normally myelinated *rag2*/(blue dots) mouse callosal-cortical strips, plotted as the percentage of mouseNG2cells versus the number ofhumanNG2cells/radial column. Across all time-points, as the total number ofhumancells in a radial column increased, the proportion of host mouse GPCs fell.***G***, The kinetics of hGPC replacement of endogenous mGPCs, as a function of time, in both *shiverer* and myelin wild-type*rag2* null hosts. In both cases, colonization by hGPCs eventually yielded the substantial replacement and, in some cases, the apparent elimination of the endogenous mouse progenitor population. Scale bars: ***A–E***, 100m.

Humane Gliazellen verdrängen Maus Glia und die Maus wird deutlich „inteligenter“.

iPSC-derived Glia „heilen“ Shiver-Phänotyp.

→ Linderung der Multiplen Sklerose; aber wie die Zellen ins Hirn bringen?

iPSC-derived Glia von Schizophrenie-Patienten machen Mäuse „schizophren“.

Instrumentalisierung des Menschen in der Forschung

Eizellspende
Samenspende
Bastozystenverbrauch
Zygotenverbrauch
Leihmütter
Gewebespenden

Eine neue Dimension der Kontingenz in der Natur

- Genexpression fluktuiert in Zellen
- Die Fluktuation ist maximal in Stammzellen
- Die Fluktuation nimmt ab während der Differenzierung
- Die Fluktuation ist minimal, aber nicht null, in ausdifferenzierten somatischen Zellen.
- Die Möglichkeit zur Transformation und Re- bzw. Transdifferenzierung bleibt.

Was Sie sich über das Detailwissen hinaus merken sollten:

- Es hat den Anschein, dass Pluripotenz eine dynamische und metastabile Eigenschaft von Zellen im Grundzustand ist.
- Makroskopische Phänomene können konstant sein, das heißt aber nicht notwendiger Weise, dass ihre mikroskopischen Bedingungen immer die selben bleiben müssen.
- Es gibt eine Grenze wo „Exaktheit“ aufhört! → Biologie, sowie andere Naturwissenschaften wahrscheinlich auch, sind keine exakte Wissenschaften. (→ siehe Kontingenzproblem in den Geisteswissenschaften)
- All das vorgetragene, ist weder wahr noch unbedingt richtig, es ist bloß die derzeit bestmögliche Sichtweise der Dinge.
- Es gibt neben technischen Einschränkungen der Forschung und ihrer Anwendung immer auch ökonomische und ethische Rahmenbedingungen die zu beachten sind.