The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
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IAEA
International Atomic Energy Agency

Workshop on Biomedical Applications of High Energy Ion Beams

Co-sponsored by: ICGEB and University of Surrey

12-16 February 2007

Venue:
Adriatico Guest House Giambiagi Lecture Hall
ICTP, Trieste, Italy

Studying Biomedical Materials

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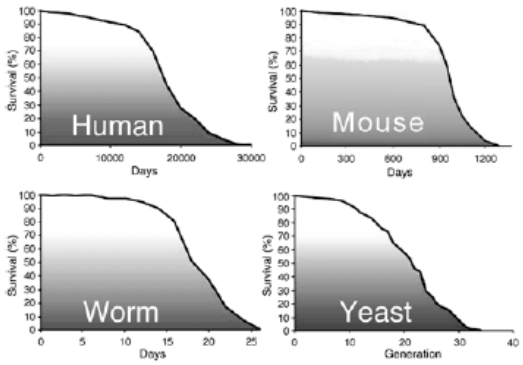
Aging

Aging is commonly characterized as a progressive, generalized impairment of function, resulting in an increasing vulnerability to environmental challenge and a growing risk of disease and death. It is also usually accompanied by a decline in fertility. Thus, aging is associated with major age-related losses in Darwinian fitness, posing the puzzle of why it has not been more efffectively opposed by natural selection.

"It is remarkable that after a seemingly miraculous feat of morphogenesis, a complex metazoan should be unable to perform the smuch simpler task of merely maintaining what is already formed" (Williams, 1957)

Characteristics of Aging

- **Increased mortality with age maturation**
- **Changes in biochemical composition of tissues** (increased adipose tissue, lipofuscin deposit, increased ECM component cross-linking, increased glycation products)



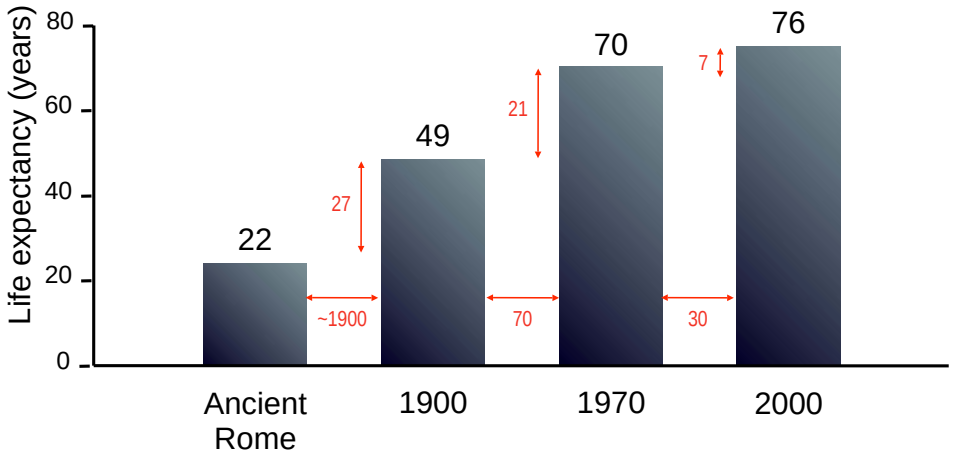
- **Progressive decrease in physiological capacities**
- **Reduced ability to adapt to environmental stimuli**
- **Increased susceptibility and vulnerability to disease** (centenarians live >90% of their lives in very good health and with high level of independence - marked morbidity compression toward the end of life)

How long shall we live?

Maximum life span for the human species (unchanged in the last 100,000 years): 125 years

The longest-lived human being is Jeanne Calment (122.5 years), died in France, in August 1997

Life expectancy at birth (in developed countries): 49 years in 1900 - 76 years in 1997



How long shall we live?

US Census Bureau Middle Series: life expectancy in 2050 will be ~82 years for both sexes in the US

US Social Security Administration: life expectancy of 78.1, 80.4 and 83.5 years for both sexes in 2066 on three alternative assumptions

G7 Industrialized Countries: life expectancy in 2050 with a maximum of 90.9 in Japan and a minimum of 82.9 years in USA

Rat: 3 years
Squirrel: 25 years
Sheep: 12 years
Turtle: 150 years
Dog: 15-30 years
Fly: 3 months

Is aging programmed genetically?

YES

Clear hereditable component in human longevity

Single-gene mutations affect life span in experimental animals

It makes evolutionary sense: aging benefits the species by preventing overcrowding: "Worn-out individuals are not only valueless for the species, but they are even harmful, for they take the place of those which are sound" (Weismann, 1889)

NO

Significant differences in longevity between human twins

Event in *C. elegans*, under controlled genetic and environmental conditions, the variation in the aging phenotype and in life span is enormous. This is very remarkable, considering that this organism is so precisely regulated that each adult has just 959 somatic cells!

In wild animal populations, in many species individuals rarely survive to ages when senescent deterioration becomes apparent, since extrinsic mortality occurs well before old age

There can have been scant opportunity to evolve genes specifically for aging, since natural selection would not normally "see" them in action

Chronic Conditions

A challenge for the 21st century

Chronic conditions are the major cause of illness, disability, and death in the United States. Almost 100 million Americans have chronic conditions and millions more will develop them as America ages. The continued growth in the number of elderly—as baby boomers age and as people live longer—will cause an increase in the number of people who are most vulnerable to and most affected by chronic conditions. Projections indicate that by 2040, almost 160 million people will have chronic conditions. The cost of medical care for Americans with chronic conditions was $470 billion in 1995. By 2040 that cost could be as high as $864 billion.[1]

MOST COMMON CHRONIC CONDITIONS

ALL AGES
- Sinusitis
- Arthritis
- Orthopedic impairments
- Hypertension
- Hay Fever

AGE 75+
- Arthritis
- Hypertension
- Hearing impairments
- Heart Disease
- Cataracts

Top 3 leading causes of death in the United States

1900's
Pneumonia & Influenza
Tuberculosis
Diarrhea & Enteritis
30% of all deaths

1990's
Heart Disease
Cancer
Stroke
60% of all deaths

Countries with highest life expectancy, 1995

Men		Women	
Japan	76.4 yrs.	Japan	82.9 yrs.
Sweden	76.2	France	82.6
Israel	75.3	Switzerland	81.9
Canada	75.2	Sweden	81.6
Switzerland	75.1	Spain	81.5
Greece	75.1	Canada	81.2
Australia	75.0	Australia	80.9
Norway	74.9	Italy	80.8
Netherlands	74.6	Norway	80.7
Italy	74.4	Netherlands	80.4

Life expectancy in the U.S. was 72.5 yrs. for men and 78.9 yrs. for women.

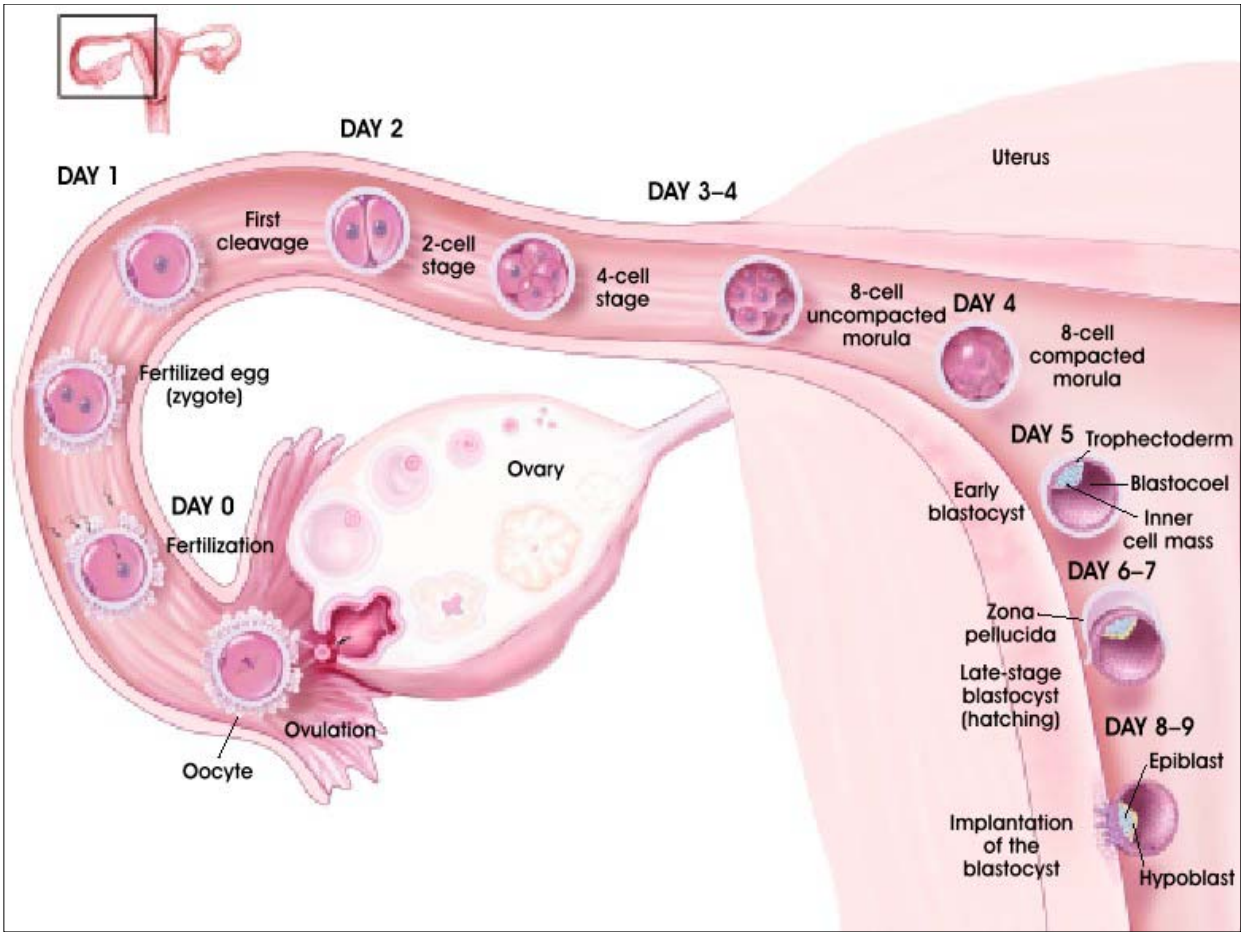
The resolution of cardiovascular disease, stroke and cancer would result only in an increase of ~15 years in life expectancy, after which aging will represent the leading cause of death

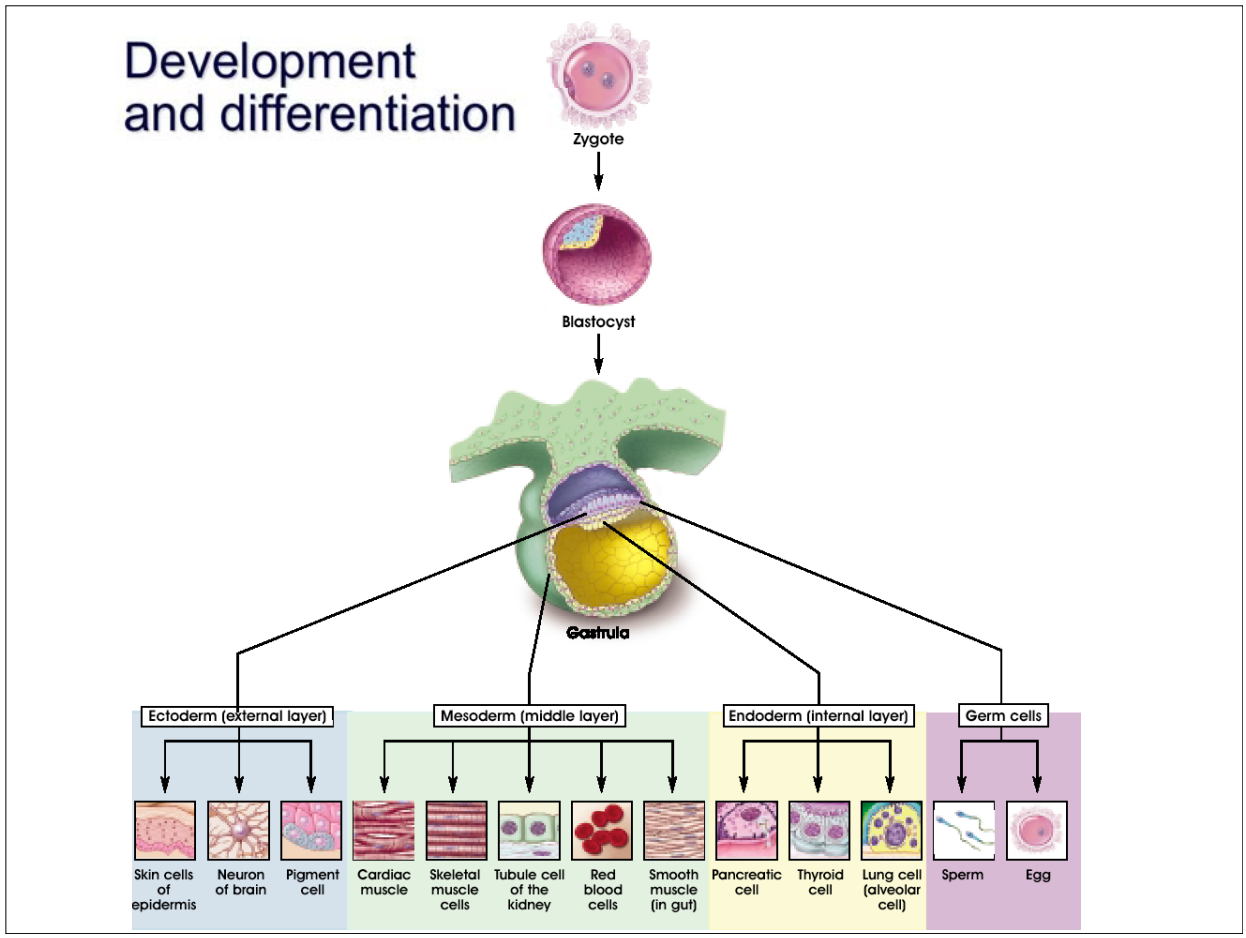
What is a stem cell?

A cell that:

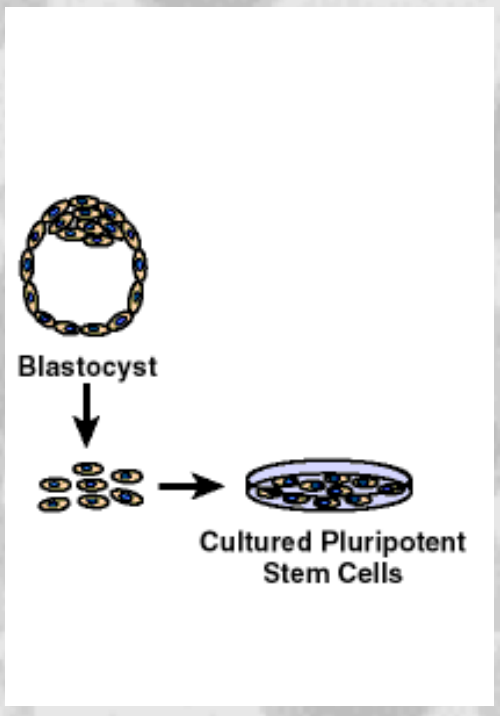
- is not differentiated
- is able to self-renewal
- can proliferate indefinitely
- can generate many cell types
- supports development, tissue homeostasis and repair

2 groups
- Embryonic stem cells (ESC)
- Adult stem cells (ASC)





Establishment in culture of pluripotent cells from mouse embryos

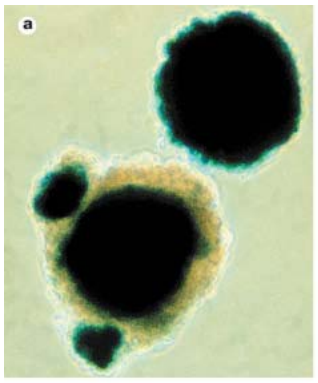


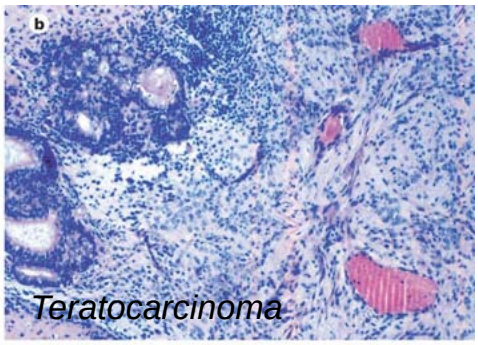
Normal caryotype!
Pluripotency!

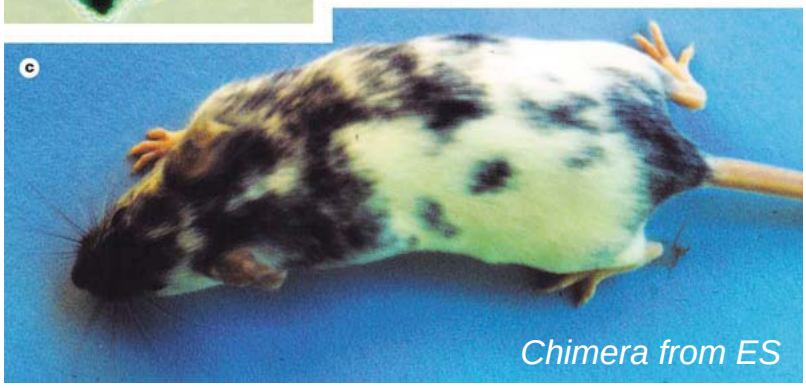
Evans MJ and Kaufman MH (1981), Nature 292, 154-156

Pluripotency of mouse embryonic stem cells (ES)

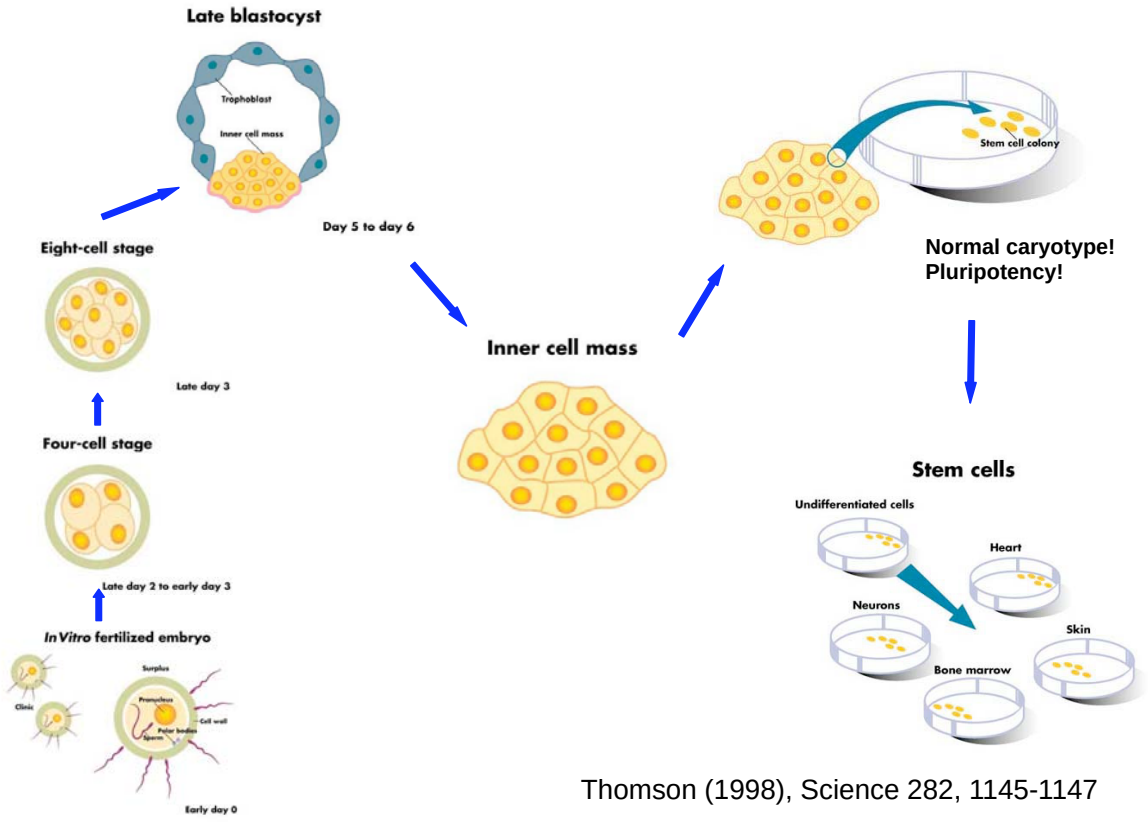
Embryoid bodies







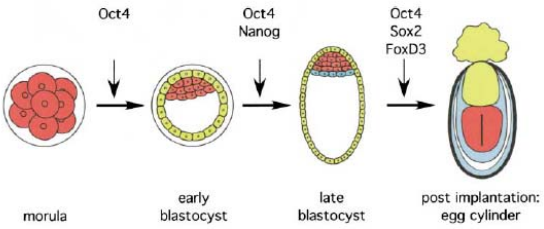
Human Embryonic Stem cells



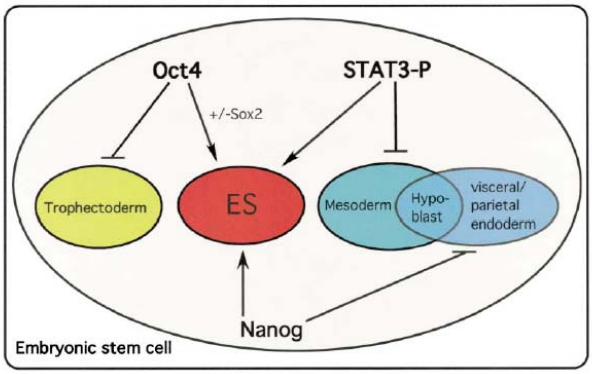
Thomson (1998), Science 282, 1145-1147

Current knowledge of what defines the potency of mouse embryonic stem cells revolves around a quartet of critical players:

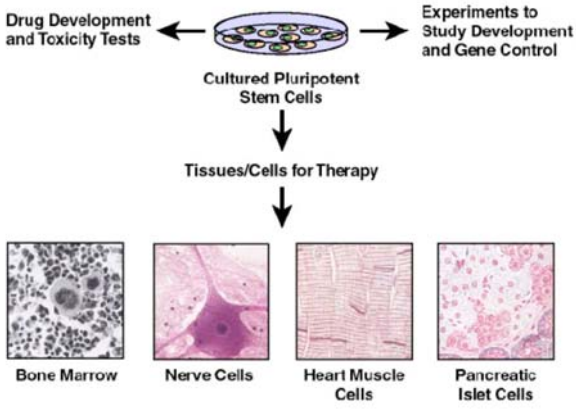
- **Oct-4**
- **Sox-2**
- **FoxD3**
- **Stat3**

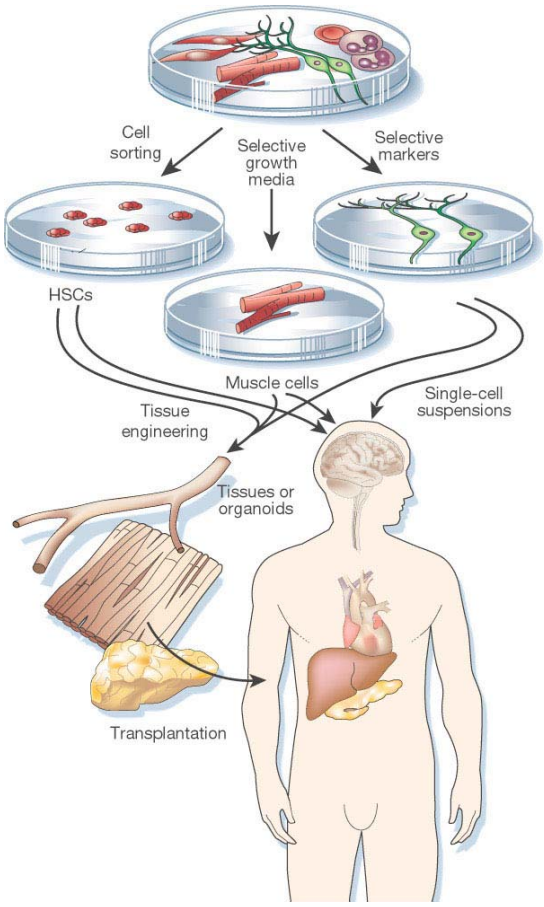


… and now also Nanog



Transplantation of ES cell-derived, differentiated cells





Directing progenitor cell along specific pathways of neuronal differentiation in a systematic manner has proved difficult, not least because the normal developmental pathways that generate most classes of CNS neurons remain poorly defined.

Cell, Vol. 110, 385–397, August 9, 2002, Copyright ©2002 by Cell Press

Directed Differentiation of Embryonic Stem Cells into Motor Neurons

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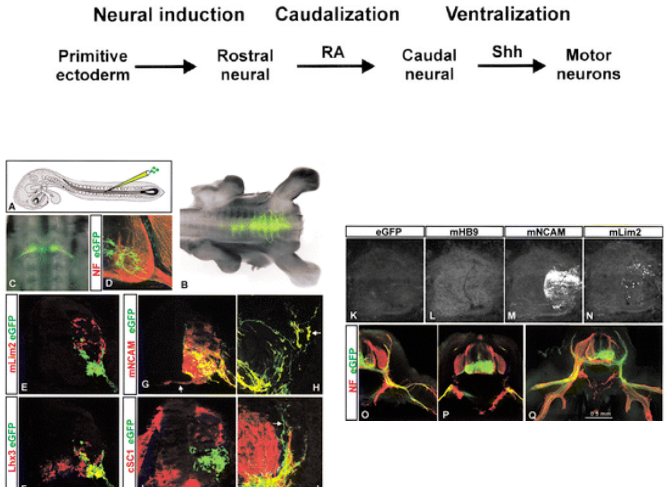

Summary

Inductive signals and transcription factors involved in motor neuron generation have been identified, raising the question of whether these developmental insights can be used to direct stem cells to a motor neuron fate. We show that developmentally relevant signaling factors can induce mouse embryonic stem (ES) cells to differentiate into spinal progenitor cells, and subsequently into motor neurons, through a pathway recapitulating that used in vivo. ES cell-derived motor neurons can populate the embryonic spinal cord, extend axons, and form synapses with target muscles. Thus, inductive signals involved in normal pathways of neurogenesis can direct ES cells to form specific classes of CNS neurons.

Early Research Shows Stem Cells Can Improve Movement in Paralyzed Mice

Researchers at Johns Hopkins University recently reported preliminary evidence that cells derived from embryonic stem cells can restore movement in an animal model of amyotrophic lateral sclerosis (ALS) [1]. This degenerative disorder, also called as Lou Gehrig's disease, progressively destroys special nerves found in the spinal cord, known as motor neurons, that control movement. Patients with ALS develop increasing muscle weakness over months to years, which ultimately leads to paralysis and death. The cause is largely unknown, and there are no effective treatments.

In this new study, the researchers used a rat model of ALS to test for possible nerve cell- restoring properties of stem cells. The rats were exposed to Sindbis virus, which infects the central nervous system and destroys the motor neurons in the spinal cord. Rats that survive are left with paralyzed muscles in their hindquarters and weakened back limbs. Scientists assess the degree of impairment by measuring the rats' movement, quantifying electrical activity in the nerves serving the back limbs, and visually judging the extent of nerve damage through a microscope.

The researchers wanted to see whether stem cells could restore nerves and improve mobility in rats. Because scientists have had difficulty sustaining stem cell lines derived from rat embryos, the investigators conducted their experiments with embryonic germ cells that John Gearhart and colleagues isolated from human fetal tissue in 1998. These cells can produce unchanged copies of themselves when maintained in culture, and they form into clumps called embryoid bodies. Under certain conditions, research has shown that the cells in the embryoid bodies begin to look and function like neurons when subjected to specific laboratory conditions [2]. The researchers had an idea that these embryoid body cells in their nonspecialized state might become specialized as replacement neurons if placed into the area of the damaged spinal cord. So they carefully prepared cells from the embryoid bodies and injected them into the fluid surrounding the spinal cord of the paralyzed rats that had their motor neurons destroyed by the Sindbis virus.

To test this idea, the researchers selected from laboratory culture dishes barely differentiated embryonic germs cells that displayed the molecular markers of neural stem cells, including the proteins nestin and neuron specific enolase. They grew these cells in large quantities and injected them into the fluid surrounding the spinal cords of partially paralyzed, Sindbis-virus-treated rats.

The response was impressive. Three months after the injections, many of the treated rats were able to move their hind limbs and walk, albeit clumsily, while the rats that did not receive cell injections remained paralyzed. Moreover, at autopsy the researchers found that cells derived from human embryonic germ cells had migrated throughout the spinal fluid and continued to develop, displaying both the shape and molecular markers characteristic of mature motor neurons. The researchers are quick to caution that their results are preliminary, and that they do not know for certain whether the treatment helped the paralyzed rats because new neurons took the place of the old, or because trophic factors from the injected cells facilitated the recovery of the rats' remaining nerve cells and helped the rats improve in their ability to use their hind limbs. Nor do they know how well this strategy will translate into a therapy for human neurodegenerative diseases like ALS. And they emphasize that there are many hurdles to cross before the use of stem cells to repair damaged motor neurons in patients can be considered. Nevertheless, researchers are excited about these results, which, if confirmed, would represent a major step toward using specialized stem cells from embryonic and fetal tissue sources to restore nervous system function.

REFERENCES

1. Kerr, D.A., Llado, J., Shamblott, M., Maragakis, N., Irani, D.N., Dike, S., Sappington, A., Gearhart, J., and Rothstein, J. (2001). Human embryonic germ cell derivatives facilitate motor recovery of rats with diffuse motor neuron injury.

2. Shamblott, M.J., Axelman, J., Wang, S., Bugg, E.M., Littlefield, J.W., Donovan, P.J., Blumenthal, P.D., Huggins, G.R., and Gearhart, J.D. (1998). Derivation of pluripotent stem cells from cultured human primordial germ cells. Proc. Natl. Acad. Sci. U. S. A. 95, 13726-13731.

The New England Journal of Medicine

TRANSPLANTATION OF EMBRYONIC DOPAMINE NEURONS FOR SEVERE PARKINSON'S DISEASE

CURT R. FREED, M.D., PAUL E. GREENE, M.D., ROBERT E. BREEZE, M.D., WEI-YANN TSAI, PH.D., WILLIAM DUMOUCHEL, PH.D., RICHARD KAO, SANDRA DILLON, R.N., HOWARD WINFIELD, R.N., SHARON CULVER, N.P., JOHN Q. TROJANOWSKI, M.D., PH.D., DAVID EIDELBERG, M.D., AND STANLEY FAHN, M.D.

ABSTRACT

Background Transplantation of human embryonic dopamine neurons into the brains of patients with Parkinson's disease has proved beneficial in open clinical trials. However, whether this intervention would be more effective than sham surgery in a controlled trial is not known.

Conclusions Human embryonic dopamine-neuron transplants survive in patients with severe Parkinson's disease and result in some clinical benefit in younger but not in older patients. (N Engl J Med 2001;344: 710-9.)

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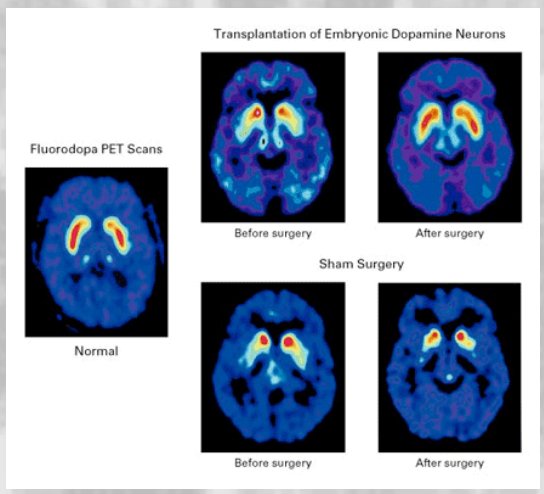
To consider the use of transplanted cells as a treatment for Parkinson's disease — whether they are pluripotent stem cells, more restricted precursors, or differentiated neurons — we must know more about their molecular composition. In addition to dopamine, such neurons probably manufacture molecules that influence neuronal proliferation, migration, differentiation, and survival. All these functions are at risk in Parkinson's disease. Also, the role of electrical-impulse activity may be important, but we know little about the functional state of the implanted cells. As the present study indicates, mere survival is not enough.



Cell therapy for Parkinson's Disease

(differentiated dopaminergic neurons do not survive after transplantation)

1980 ***Transplantation of dopamine-producing cells from patient's own adrenal glands***

1982 ***Transplantation of fetal tissue into the damaged area of the brains in rats and monkeys models of Parkinson's Disease***

1985 ***Fetal tissue transplantation in humans***

1995 ***NIH funding for two double blind, placebo control clinical trials of fetal tissue transplantation***

Cell therapy for diabetes: burden of the disease

Diabetes is the seventh leading cause of death in the US today (200.000 deaths reported each year)

Excess of glucose is responsible for most of the complications - blindness, kidney failure, heart disease, stroke, neuropathy and amputations

Type 1: juvenile-onset diabetes, autoimmune destruction of beta-cells
Type 2: adult-onset, familiar, insulin-resistance

No cure available
Support therapy: insulin (type 1), diet, exercise, oral medications (type 2)
Whole organ transplant requires strong immunosuppression (only in combination with kidney transplant)

The New England Journal of Medicine

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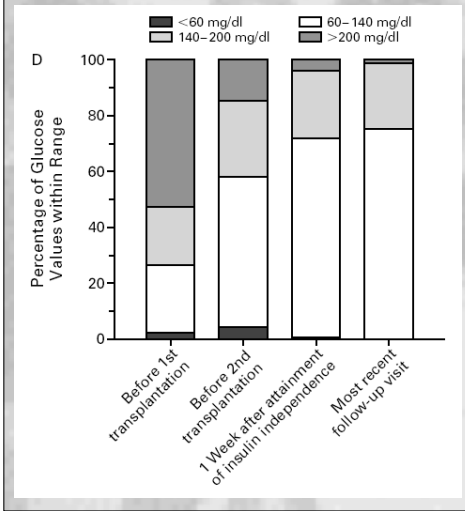
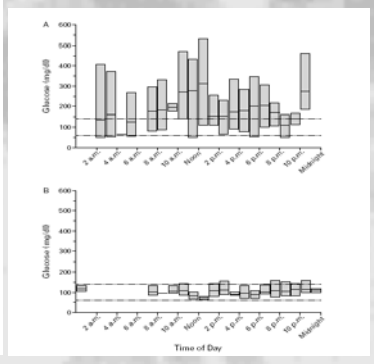
VOLUME 343 JULY 27, 2000 NUMBER 4

ISLET TRANSPLANTATION IN SEVEN PATIENTS WITH TYPE 1 DIABETES MELLITUS USING A GLUCOCORTICOID-FREE IMMUNOSUPPRESSIVE REGIMEN

A.M. JAMES SHAPIRO, M.B., B.S., JONATHAN R.T. LAKEY, PH.D., EDMOND A. RYAN, M.D., GREGORY S. KORBUTT, PH.D., ELLEN TOTH, M.D., GARTH L. WARNOCK, M.D., NORMAN M. KNETEMAN, M.D., AND RAY V. RAJOTTE, PH.D.

Patient and Procedure No.	Age of Donor	Duration of Cold Ischemia		Total Beta-Cell Mass per Transplant*
		From cross-clamping to islet isolation	From cross-clamping to implantation	
	yr	hr		×10⁻⁶
Patient 1				
1	35	4.0	7.5	102.2
2	41	9.5	14.5	192.4
Patient 2				
1	71	1.5	8.0	173.6
2	17	8.5	18.0	262.5
Patient 3				
1	48	3.0	11.4	113.8
2	22	5.0	14.2	42.9
Patient 4				
1	65	2.0	7.0	60.2
2	38	2.5	10.3	181.3
3§	42	5.0	43.0	139.1
3§	39	3.5	21.0	193.2
Patient 5				
1	54	6.5	13.3	100.6
2	57	1.5	7.0	166.2
Patient 6				
1	51	6.0	11.5	101.5
2	44	13.0	18.4	31.1
Patient 7				
1	55	5.0	10.5	50.1
2	41	1.0	6.5	197.8
Mean (±SD) values	45.0±14	4.8±3	13.9±9	132±67

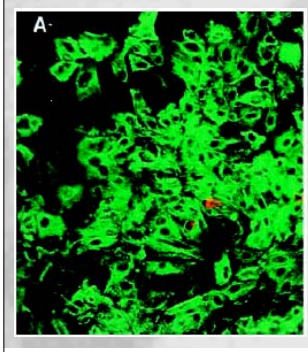




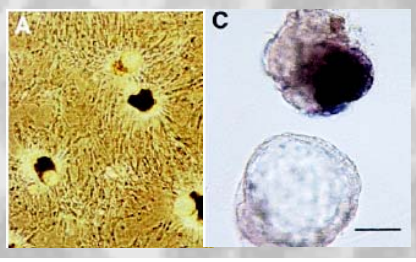
Important limits:
- **2 donors per transplant**
- **hystocompatibility**
- **early explant (max 8 hr)**

In vitro cultivation of human islets from expanded ductal tissue

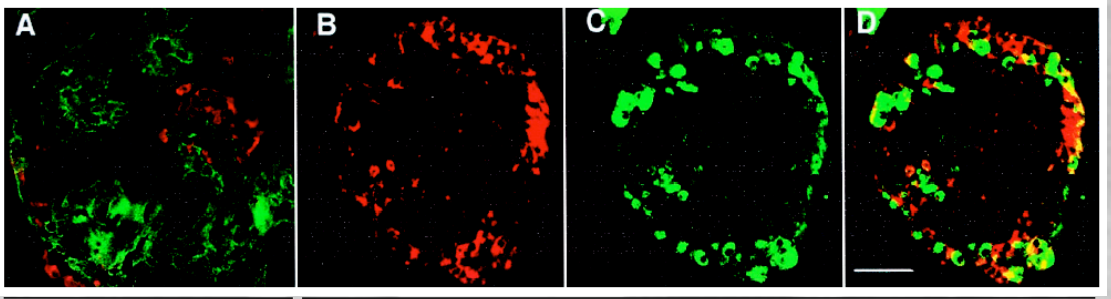
Susan Bonner-Weir*, Monica Taneja, Gordon C. Weir, Krystyna Tatarkiewicz, Ki-Ho Song, Arun Sharma, and John J. O'Neil



Primary ductal cells
green = cytokeratin
red = insulin



CHIBs rising from a monolayer of ductal cells
(dithizone staining)



green = cytokeratin
red = insulin

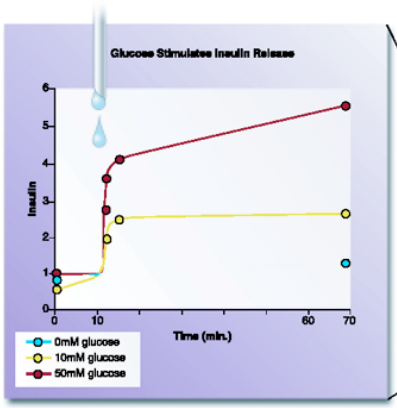
red = insulin
green = non beta-cell hormones (glucagons, somatostatin, PP)

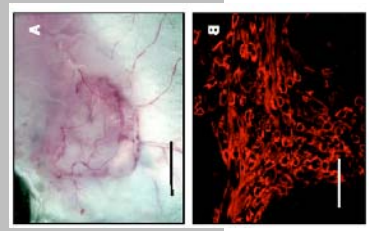
ES cells transplantation for diabetes

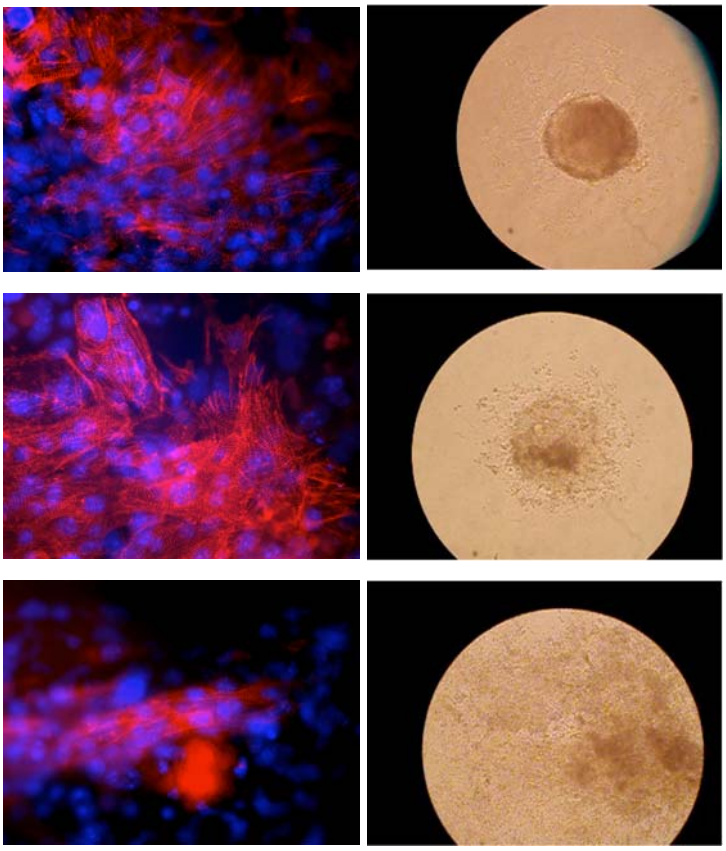
Differentiation of Embryonic Stem Cells to Insulin-Secreting Structures Similar to Pancreatic Islets

Nadya Lumelsky,[1] Olivier Blondel,[1,3] Pascal Laeng,[2,4] Ivan Velasco,[1] Rea Ravin,[1] Ron McKay[1]*

www.sciencemag.org SCIENCE VOL 292 18 MAY 2001



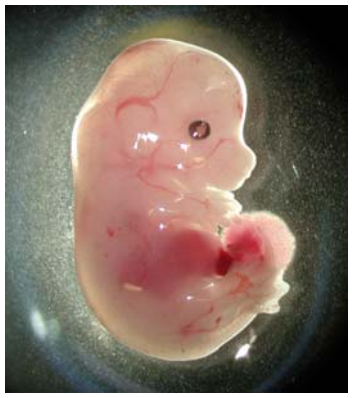


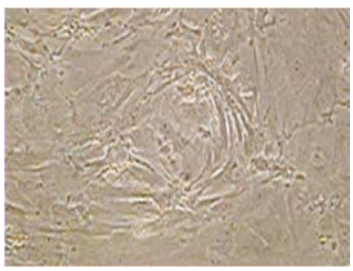


Embryonic cardiomyocytes

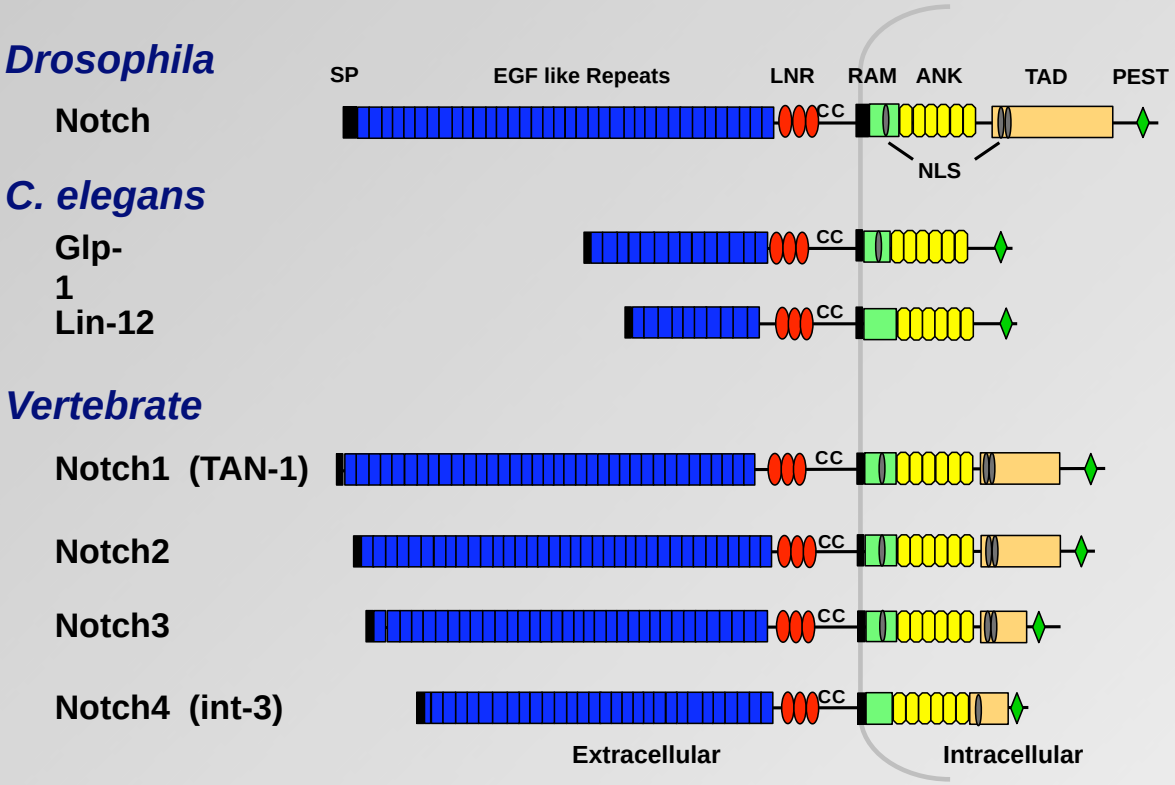
alpha-actinin

Fetal cardiomyocytes

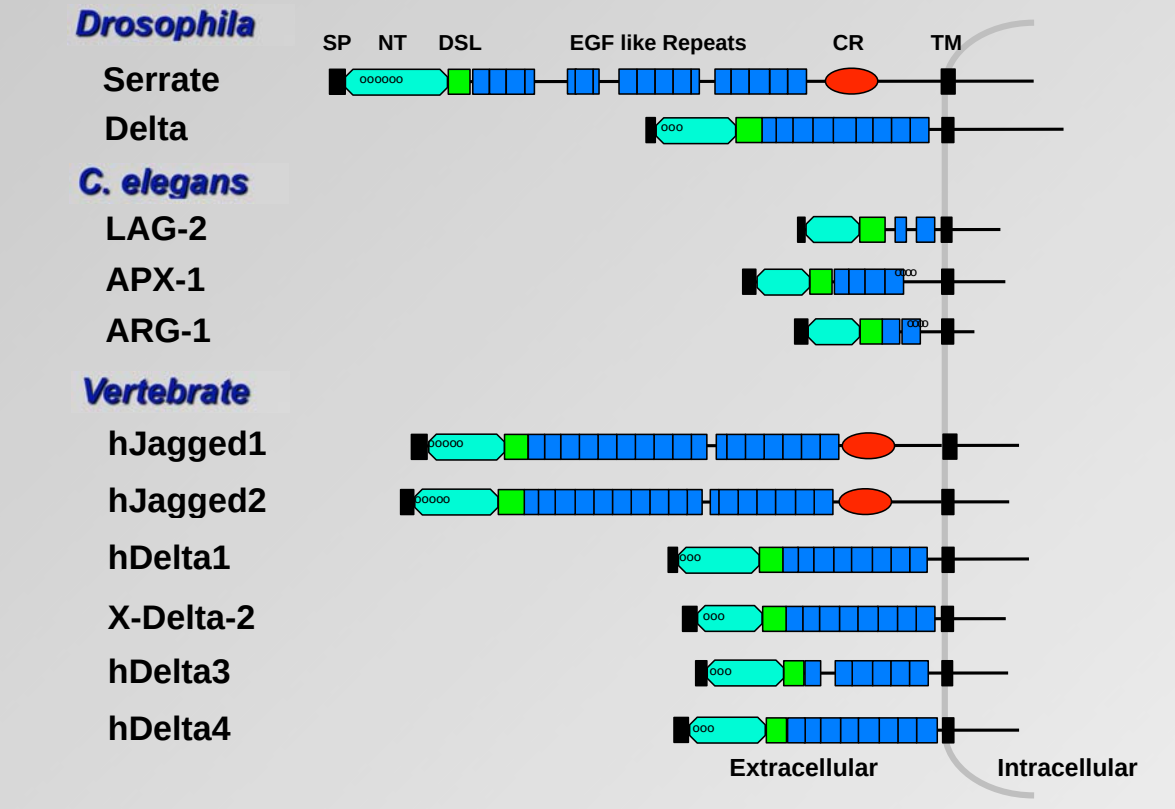




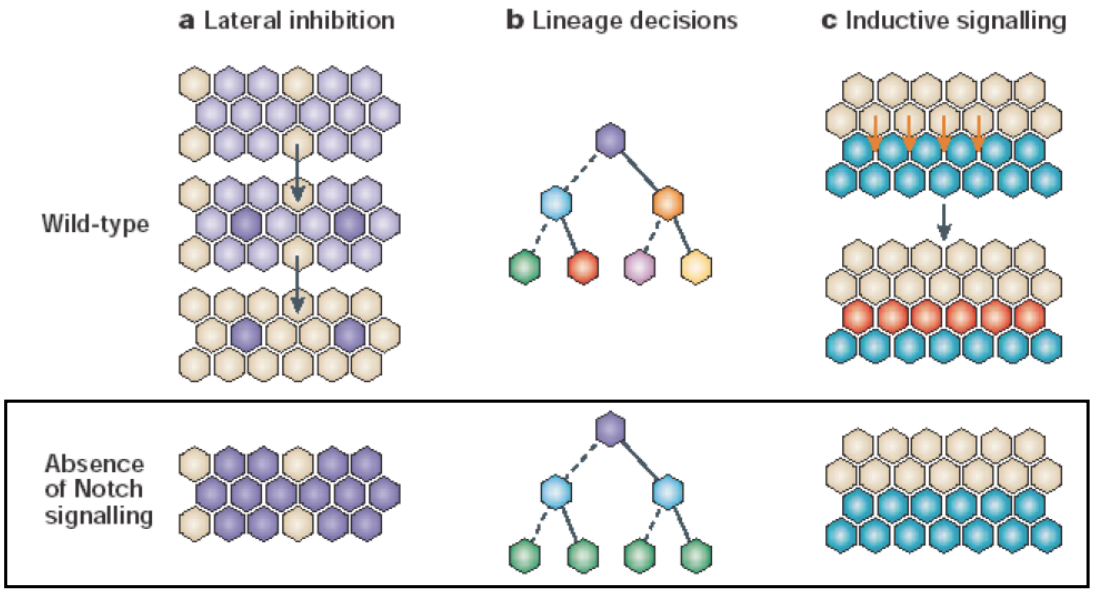
The Notch family of receptors



The DSL family of ligands



Distinct Function modes of Notch signalling

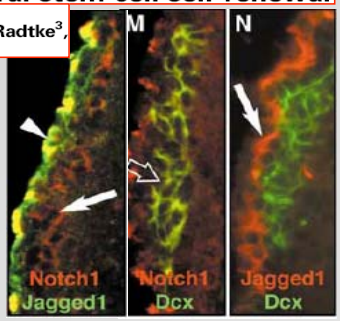


Nicola Haines and Kenneth D. Irvine *NATURE REVIEWS* | *MOLECULAR CELL BIOLOGY* **2003 4:786**

Jagged1 signals in the postnatal subventricular zone are required for neural stem cell self-renewal

Yves Nyfeler[1], Robert D Kirch[1], Ned Mantei[2], Dino P Leone[2], Freddy Radtke[3], Ueli Suter[2] and Verdon Taylor[1,*]

The EMBO Journal (2005), 1–12



Jagged-1/Notch-1

Activation of Notch signaling pathway precedes heart regeneration in zebrafish

Ángel Raya*†, Christopher M. Koth*†, Dirk Büscher*†, Yasuhiko Kawakami*†, Tohru Itoh*†, R. Marina Raya*, Gabriel Sternik*, Huai-Jen Tsai‡, Concepción Rodríguez-Esteban*, and Juan Carlos Izpisúa-Belmonte*§

PNAS | September 30, 2003 | vol. 100 | suppl. 1 | 11889–11895

MicroRNA1 influences cardiac differentiation in *Drosophila* and regulates Notch signaling

Chulan Kwon*†§, Zhe Han‡§, Eric N. Olson‡, and Deepak Srivastava*†¶

18986–18991 | PNAS | December 27, 2005 | vol. 102 | no. 52

Notch promotes epithelial-mesenchymal transition during cardiac development and oncogenic transformation

Luika A. Timmerman,[1,6] Joaquín Grego-Bessa,[2,5,6] Angel Raya,[3] Esther Bertrán,[2] José María Pérez-Pomares,[4] Juan Díez,[2] Sergi Aranda,[2] Sergio Palomo,[2] Frank McCormick,[1] Juan Carlos Izpisúa-Belmonte,[3] and José Luis de la Pompa[2,5,7]

GENES & DEVELOPMENT 18:99–115 © 2004 by Cold Spring Harbor Laboratory Press ISSN 0890-9369/04; www.genesdev.org

Mutations in *NOTCH1* cause aortic valve disease

Vidu Garg[1,3], Alecia N. Muth[1†], Joshua F. Ransom[1†], Marie K. Schluterman[1], Robert Barnes[3,4], Isabelle N. King[1,3†], Paul D. Grossfeld[5] & Deepak Srivastava[1,2,4,3†]

Vol 437|8 September 2005|doi:10.1038/nature03940

Serrate and Notch specify cell fates in the heart field by suppressing cardiomyogenesis

Development 127, 3665-3676 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
DEV4379

Melissa S. Rones, Kelly A. McLaughlin, Michael Raffin and Mark Mercola*

Hypoxia Requires Notch Signaling to Maintain the Undifferentiated Cell State

Maria V. Gustafsson, Xiaowei Zheng, Teresa Pereira, Katarina Gradin, Shaobo Jin, Johan Lundkvist, Jorge L. Ruas, Lorenz Poellinger, Urban Lendahl,* and Maria Bondesson[1]

Developmental Cell, Vol. 9, 617–628, November, 2005,

Development 133, 1625-1634 (2006) doi:10.1242/dev.02344

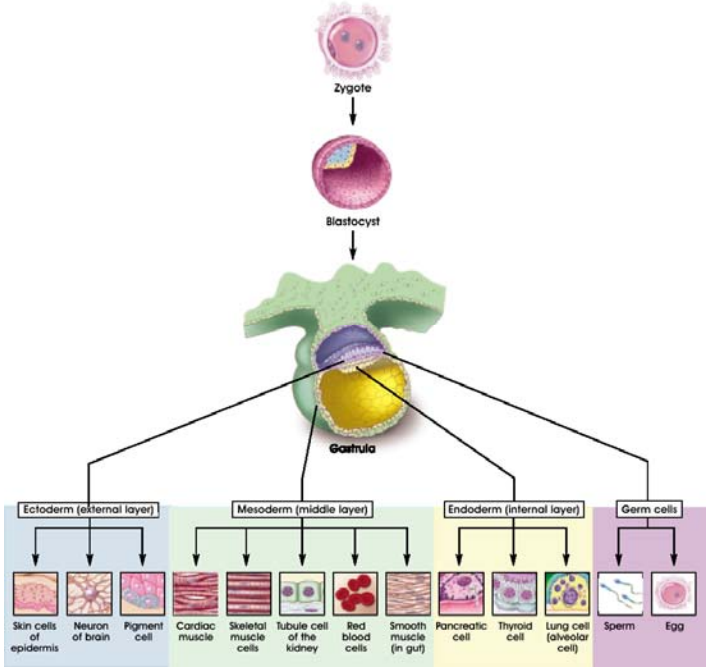
Activation of Notch1 signaling in cardiogenic mesoderm induces abnormal heart morphogenesis in mouse

Yusuke Watanabe[1,*,†], Hiroki Kokubo[1,2,*], Sachiko Miyagawa-Tomita[3], Maho Endo[1], Katsuhide Igarashi[4], Ken ichi Aisaki[4], Jun Kanno[4] and Yumiko Saga[1,2,‡]

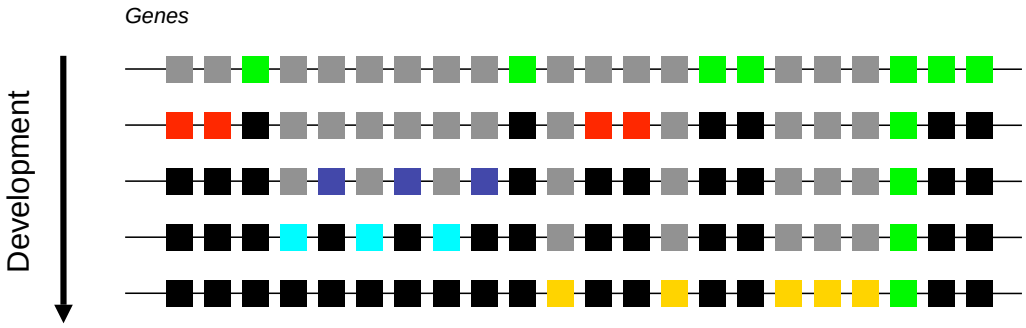
Using Stem Cells for human therapy: the problems

- ***Imprinted genes***
- ***Aneuploidy:*** ***in primates the process of removing the resident nucleus causes molecules associated with the centrosome to be lost as well***
- ***Somatic mutations***
- ***Political controversy***

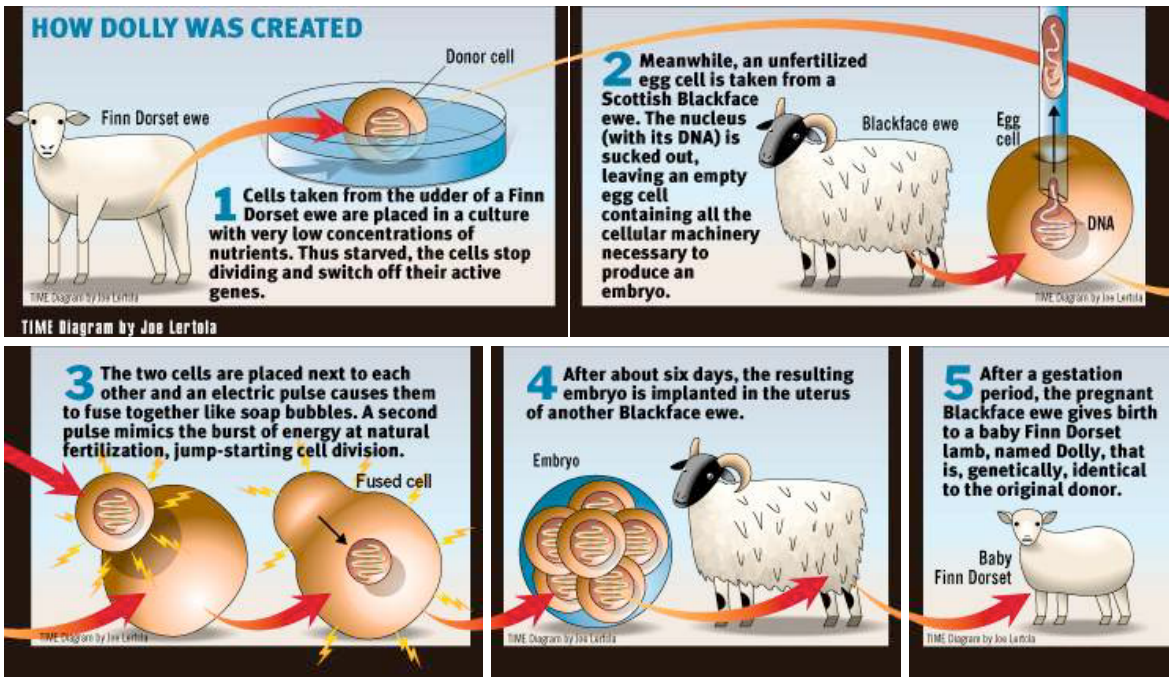
Development and differentiation



Genetic programs



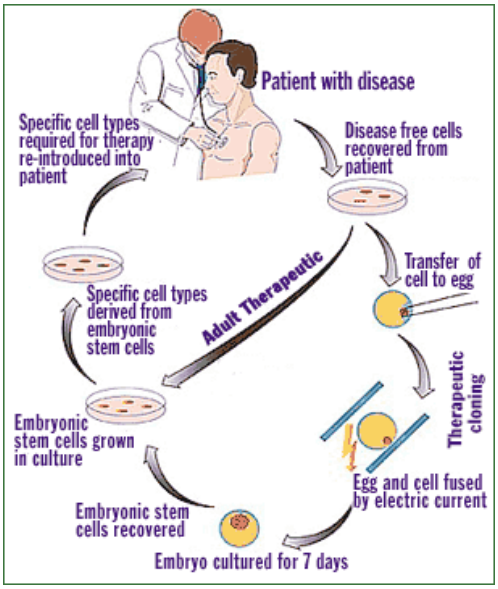
Cloning



Applications of cloning

- Treatment of human infertility NO!
- Transgenic animals for drug production
- Genetic rescue of endangered mammals
- Animal organs for human xenotransplantation
- Therapeutic cloning for human stem cell therapy
- Human tissue and organ engineering
- Rescue of genetic defect by ex vivo gene therapy

Therapeutic cloning



Please Don't Call It Cloning!

Bert Vogelstein et al., Science 2002

THE CRUCIAL DIFFERENCES

	Nuclear transplantation	Human reproductive cloning
End product	Cells growing in a petri dish	Human being
Purpose	To treat a specific disease of tissue degeneration	Replace or duplicate a human
Time frame	A few weeks (growth in culture)	9 months
Surrogate mother needed	No	Yes
Sentient human created	No	Yes
Ethical implications	Similar to all embryonic cell research	Highly complex issues
Medical implications	Similar to any cell-based therapy	Safety and long-term efficacy concerns

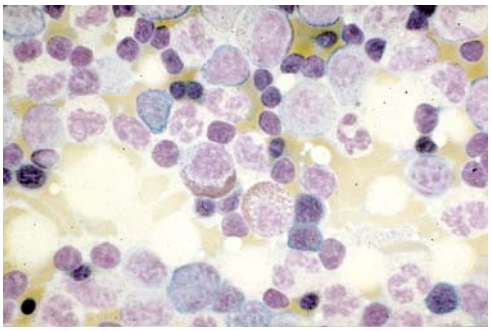
What is a stem cell?

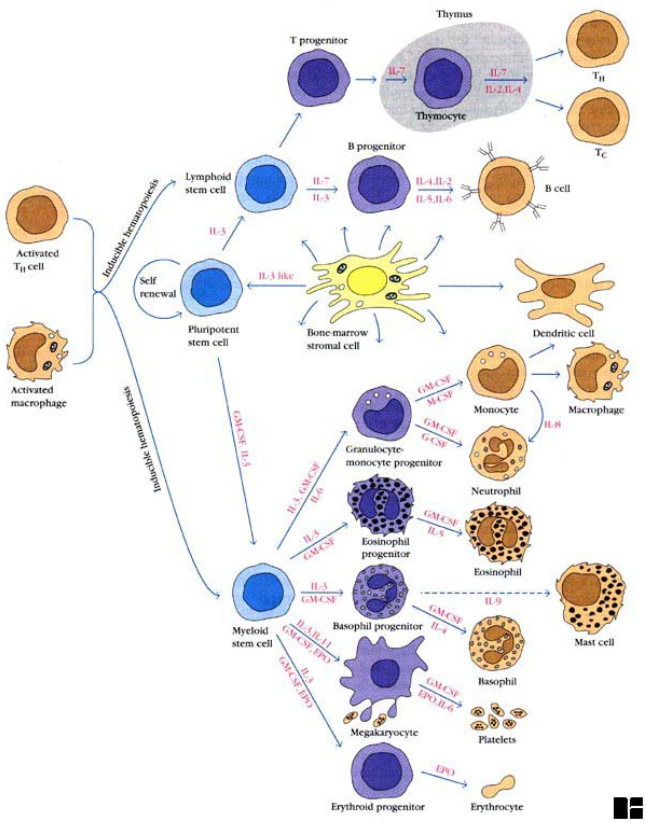
A cell that:

- is not differentiated
- is able to self-renewal
- can proliferate indefinitely
- can generate many cell types
- supports development, tissue homeostasis and repair

2 groups
- Embryonic stem cells (ESC)
- Adult stem cells (ASC)

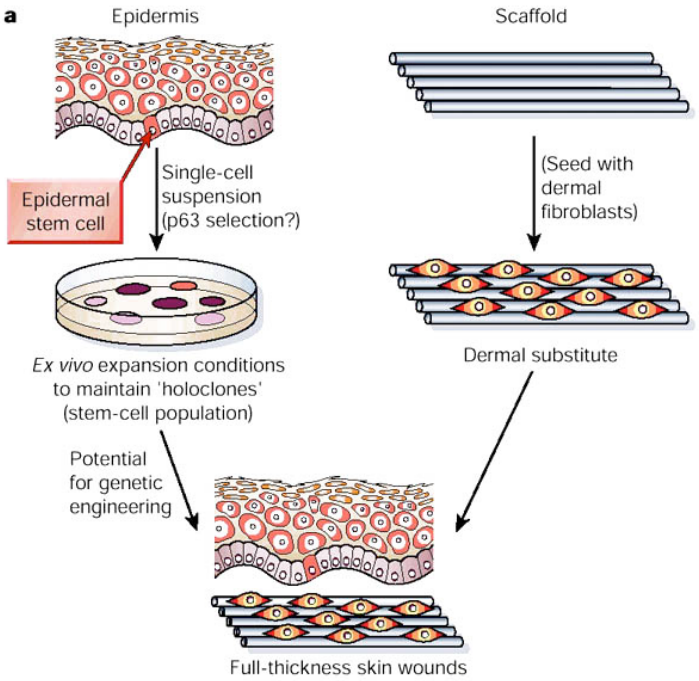
Hematopoiesis





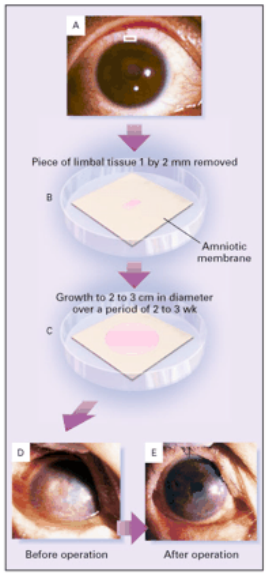
Skin autografts produced by stem cell derived keratinocytes

Holoclones: ***product of a true stem cell (>140 ds)***
Meroclones: ***a population of transient amplifying cells***
Paraclones: ***senescent or differentiating progenitors***



Long term success of skin autografts depends on:

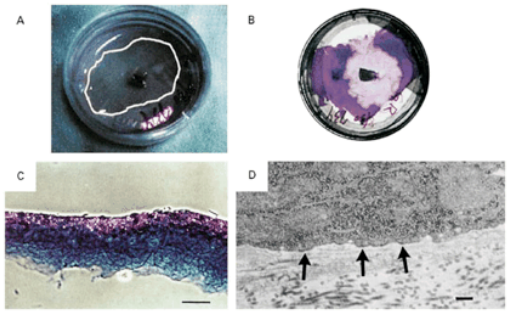
- appropriate replenishment of stem cells
- nature of the dermis-like substrate

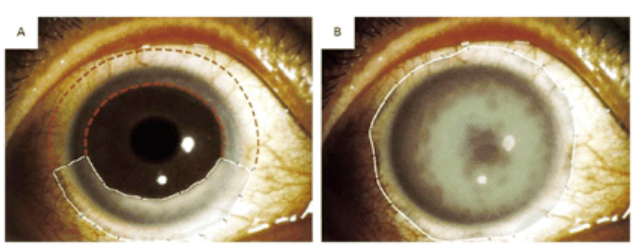


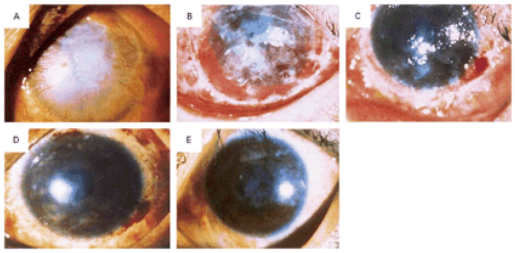
The New England Journal of Medicine

RECONSTRUCTION OF DAMAGED CORNEAS BY TRANSPLANTATION OF AUTOLOGOUS LIMBAL EPITHELIAL CELLS

RAY JUI-FANG TSAI, M.D., LIEN-MIN LI, B.S., AND JAN-KAN CHEN, PH.D.







Sources of adult stem cells

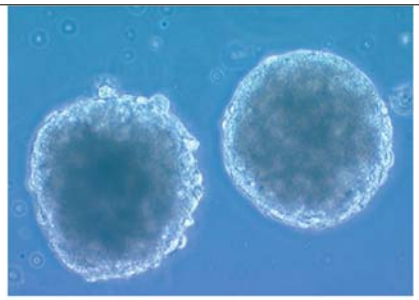
Bone marrow:	*HSC and MSC*
Peripheral blood:	*HSC, hemangioblast?*
Brain and spinal cord:	*NSC*
Skin:	*bulge zone cells, SKP in the dermis*
Liver:	*oval cells*
Pancreas:	*ductal stem cells*
Eye:	*corneal and retinal stem cells*
Skeletal muscle:	*satellite cells and SP*

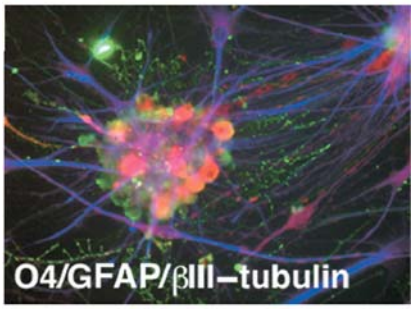
Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain

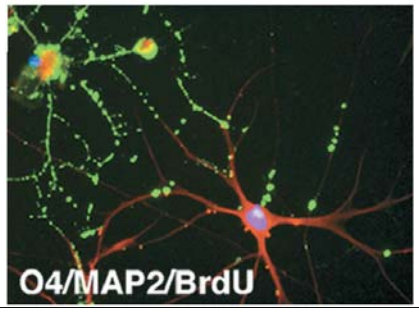
Marta C. Nunes[1], Neeta Singh Roy[1], H. Michael Keyoung[1], Robert R. Goodman[2], Guy McKhann II[2], Li Jiang[3], Jian Kang[3], Maiken Nedergaard[3] & Steven A. Goldman[1]

[1]Department of Neurology and Neuroscience, Cornell University Medical College, New York, New York, USA
[2]Department of Neurosurgery, Columbia University College of Physicians and Surgeons, New York, New York, USA
[3]Department of Anatomy and Cell Biology, New York Medical College, Valhalla, New York, USA
M.C.N. and N.S.R. contributed equally to this work.
Correspondence should be addressed to S.A.G.; e-mail: sgoldm@mail.med.cornell.edu

Published online 10 March 2003; doi:10.1038/nm837





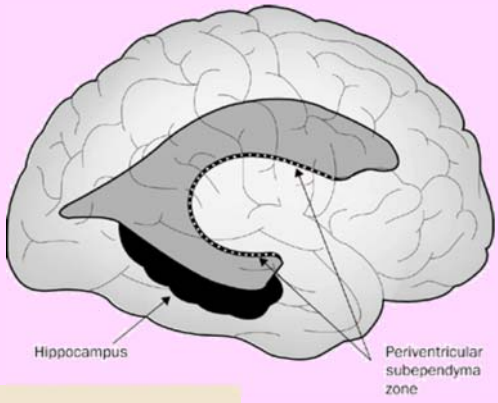


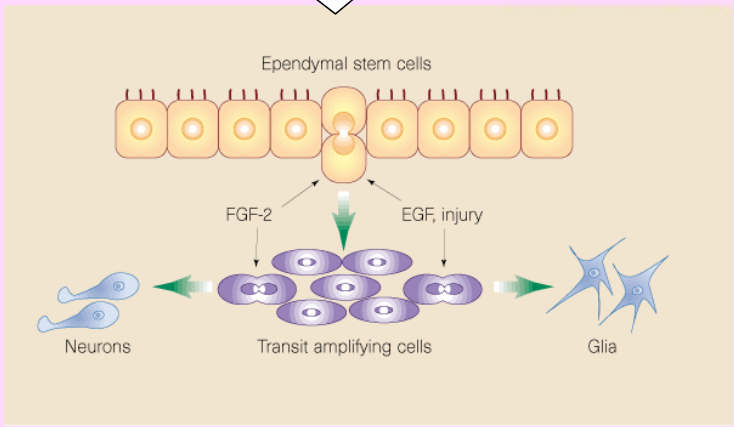
O4 = oligodendrocytes, green

GFAP = astrocytes, blue

βIII-tubulin, MAP = neurons, red

Brain marrow
location of Neural Stem Cells

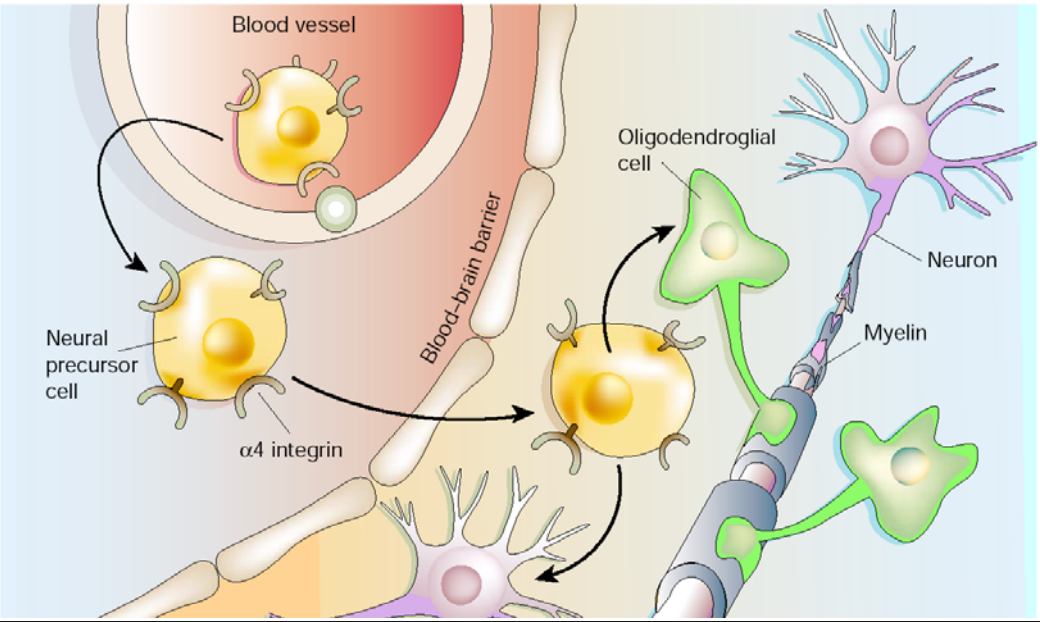




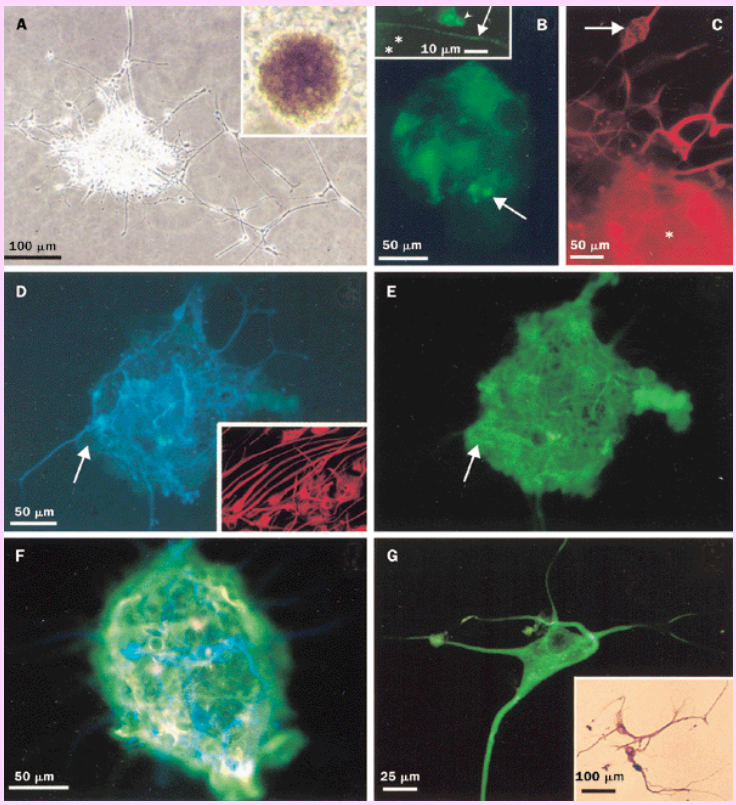
articles

Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis

Stefano Pluchino*, Angelo Quattrini†‡, Elena Brambilla*, Angela Gritti§, Giuliana Salani*, Giorgia Dina†, Rossella Galli§, Ubaldo Del Carro‡, Stefano Amadio‡, Alessandra Bergami*, Roberto Furlan*‡, Giancarlo Comi‡, Angelo L. Vescovi§ & Gianvito Martino*‡



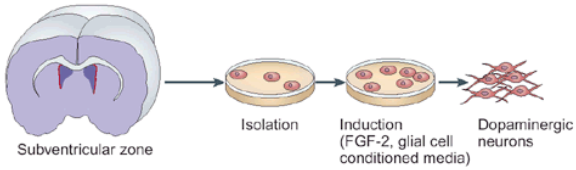
Human neurosphere clones

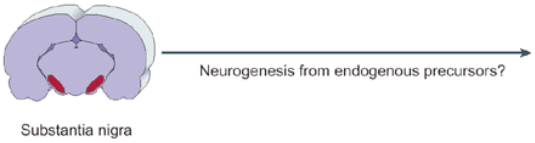


A neurosphere is a tissue-culture-generated clone of cells in different states of differentiation, all presumed to arise from a single multipotent stem/progenitor cell

- ***A. Neurosphere on laminin*** ***(inset: semi-solid media)***
- *B. α-nestin*
- *C. α-vimentin*
- *D. α-GFAP*
- *E. α-βIII tubulin*
- *F. α-GFAP + α-βIII tubulin*
- *G. De novo generated neuron* ***(α-β III tubulin and peroxidase)***

Neural stem cells from adult brain

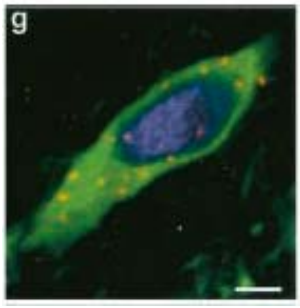




Evidence for neurogenesis in the adult mammalian substantia nigra

Ming Zhao*†, Stefan Momma‡¶, Kioumars Delfani*, Marie Carlén‡, Robert M. Cassidy‖, Clas B. Johansson‡, Hjalmar Brismar‖, Oleg Shupliakov*, Jonas Frisén‡**, and Ann Marie Janson*†§

Departments of *Neuroscience, ‡Cell and Molecular Biology, Medical Nobel Institute, and §Woman and Child Health, Karolinska Institute, SE-171 77 Stockholm, Sweden



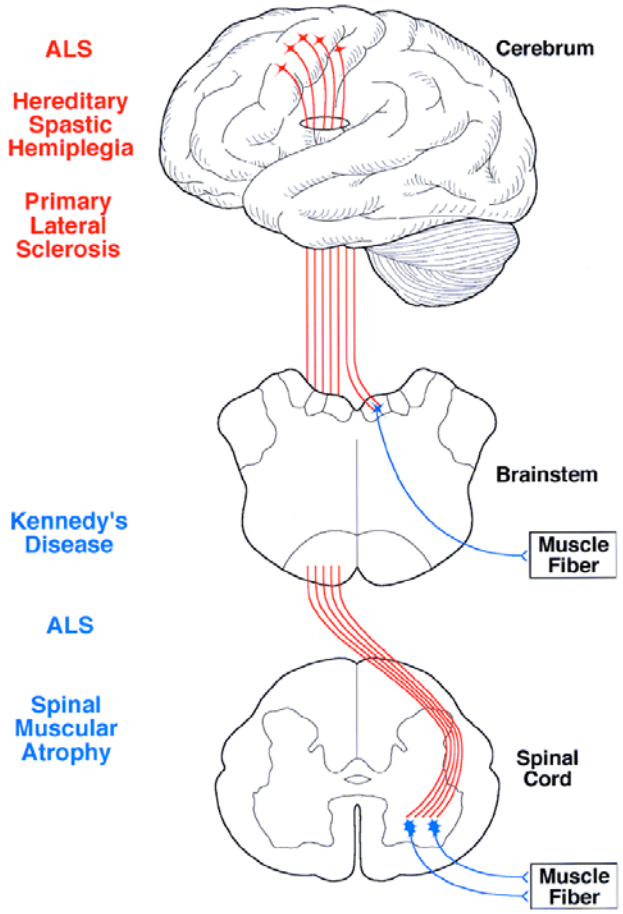
There is a continuous formation of dopaminergic neurons in the adult mouse substantia nigra, and the rate of neurogenesis can double after a lesion of the dopamine system

Other investigators observed only a glial response and failed to detect neurogenesis following dopaminergic lesions (Lie, J Neurosci 2002, Mao, Dev Brain Res 2001) --> in this study evidence of neurogenesis was mainly based on BrdU incorporation, which may have also other explanations

PNAS | June 24, 2003 | vol. 100 | no. 13 | 7925–7930

Motor neuron diseases

involve lesions in one or both components of a two-neuron pathway



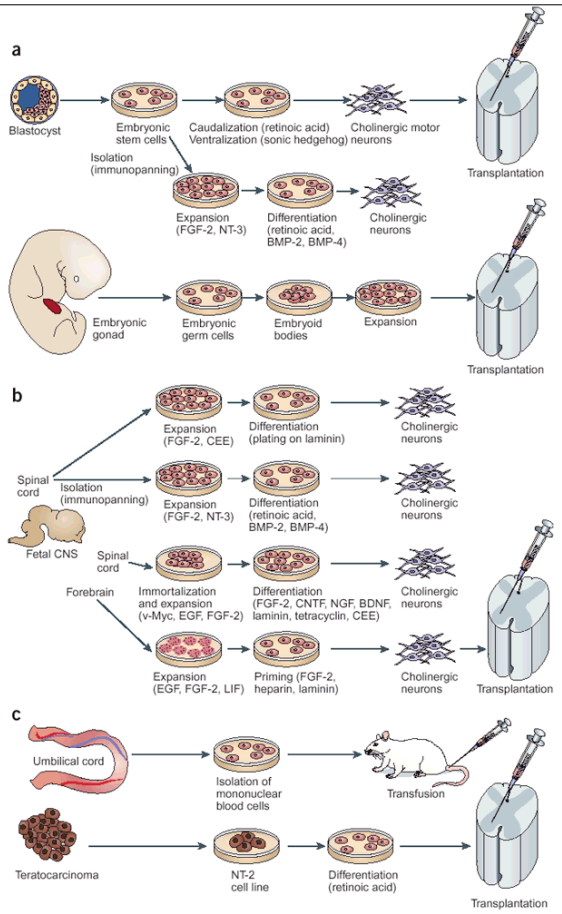
Amyotrophic lateral sclerosis
(Lou Gehrig's disease)

- Lower and upper motor degeneration
- Onset at 40-50 years
- Respiratory failure within 2-5 years
- Deterioration can be slowed by riluzole (glutamate-blocking drug) and antioxidant vitamins - but modest/no improvement
- 10% genetic forms: earlier onset, Lewy body inclusions and spinocerebellar degeneration

Stem cell therapy for amyotrophic lateral sclerosis

In its common form, ALS is characterized by progressive dysfunction and degeneration of motor neurons in cerebral cortex, brain stem and spinal cord. Muscle weakness progresses rapidly and causes death within a few years.

To have long-term value, stem cell therapy must restore function of both upper and lower motor neurons



Current approaches to tissue engineering

Stem cell-based tissue engineering

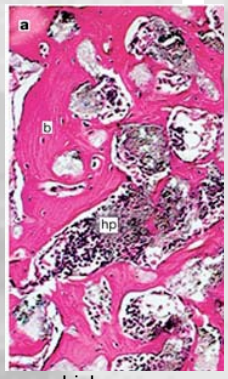
- Blood vessels
- Bone*
- Cartilage
- Cornea *
- Dentin
- Heart muscle
- Liver
- Pancreas
- Nervous tissue *
- Skeletal muscle
- Skin *

Non stem cell-based tissue engineering

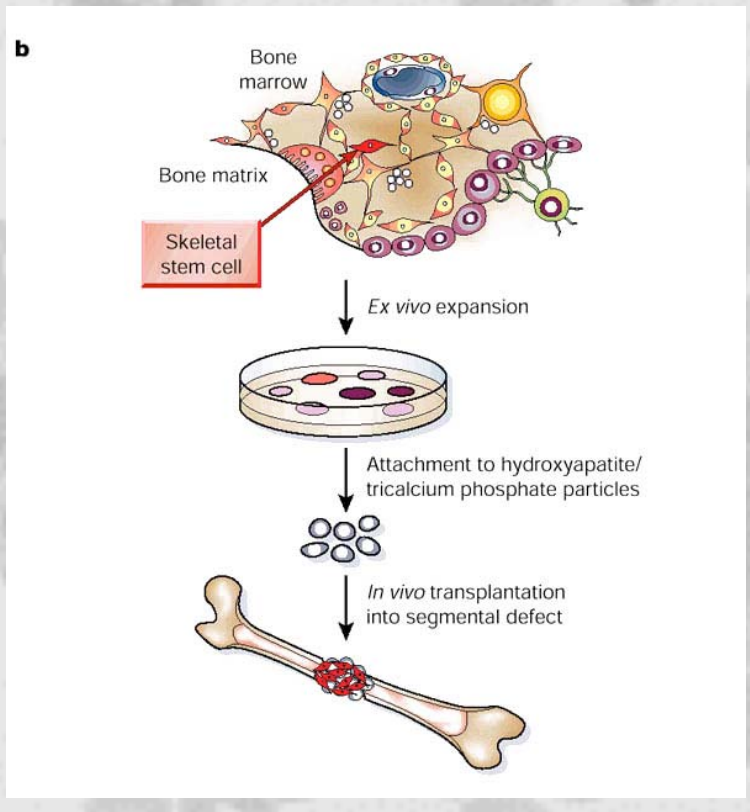
- Bladder
- Cartilage (ear, nose and joints) *
- Heart valves
- Intestine
- Kidney
- Meniscus
- Oral mucosa
- Salivary gland
- Trachea
- Ureter
- Urethra

**** in clinical trials or clinical observational studies***

Three-dimensional bone regeneration



v. vehicle
b. bone
hp. stroma composed of adipocytes and reticular cells that support hemopoiesis



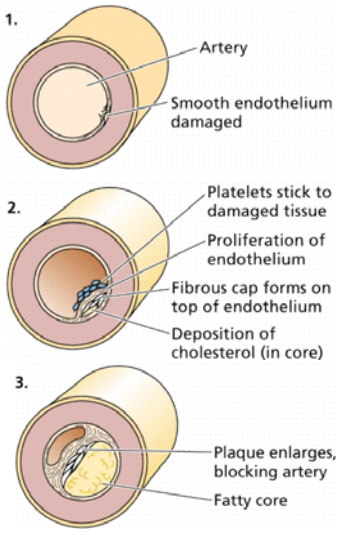
Cardiovascular disorders

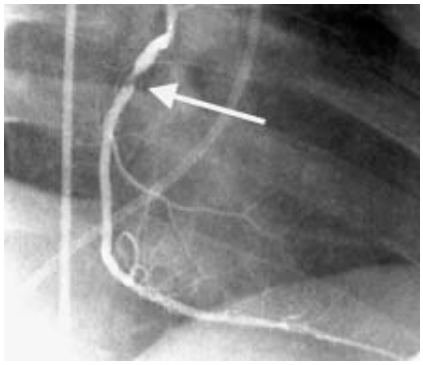
Commonest cause of death in developed countries (more than 1 person out of 3 dies because of cardiovascular disorders, including myocardial infarction (49%) or stroke (28%)

Over 20% males under 60 years have ischemic heart disease

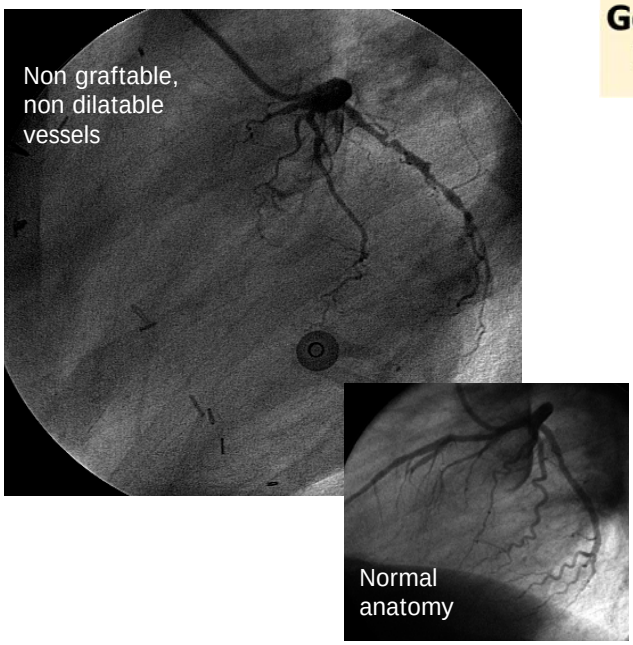
In Europe, about 600.000 people die of myocardial infarction every year, with an incidence of 1:6 among men and 1:17 among women

More than 50% of patients with ischemic cardiomyopathy die within 4 years from the beginning of symptoms, independent from therapy





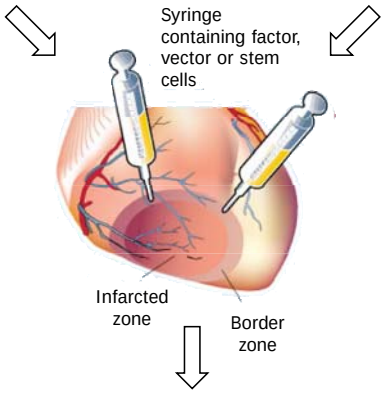
New treatments for myocardial ischemia



Recombinant proteins
Which factors?

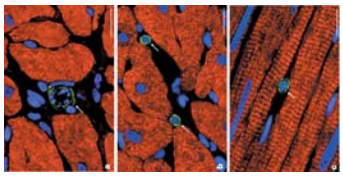
Gene Therapy
Relevant genes?
Which vector?

Cell Therapy
Source??



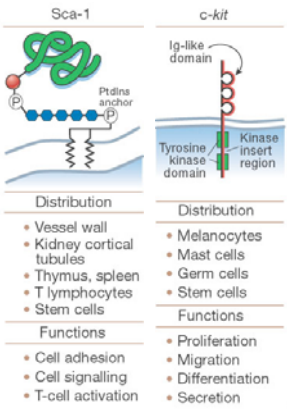
Neoangiogenesis
Myocardial protection
Myocardial regeneration

Cardiac stem cells: do they exist?



Supplementary Table 2. Comparison of isl1$^+$ cardioblasts, cardiac sca-1$^+$ cells and cardiac side population (SP) cells.

	isl1$^+$ cardioblasts	cardiac sca-1$^+$ cells[1]	cardiac SP cells[2]
1. Hoechst 33342 dye efflux	Hoechst dye excluding cells: 4.5%	Hoechst dye excluding cells: 3.6%	Hoechst dye excluding cells: 100%
2. Marker expression	sca1 negative CD31 negative c-kit negative Nkx2.5 positive GATA4 positive myocytic marker negative	sca1 positive CD31 positive c-kit negative Nkx2.5 negative GATA4 positive myocytic marker negative	sca1 positive CD31 negative c-kit positive (low) Nkx2.5 negative GATA4 negative myocytic marker negative
3. *in vivo* localization	• outflow tract • free wall of atria • intra-atrial septum • conus muscle • right ventricle	• adjacent to basal lamina • no preferred heart region	• not determined
4. Progenitor identity determined by lineage tracing	• isl1 identifies cardiac progenitor cells • established embryonic lineage marker for the heart	• sca-1 surface marker used for cell purification • no cardiac lineage marker	• Abcg2 activity used for Hoechst dye efflux • no cardiac lineage marker
5. Myocytic differentiation *in vitro*	‹‹-actinin expression with sarcomeric structure : 22% cardiac troponin T : 25%	‹‹-actin expression without sarcomeric structure : 4.6% cardiac troponin I : 2.8%	‹‹-actinin expression without sarcomeric structure : % not determined
6. Myocytic differentiation *in vivo* after cell transplantation	not determined	ischemia/reperfusion injury: ~1.5% differentiation ~1.5% cell fusion	not determined
7. Functional evaluation of *in vitro* differentiated cells	• Ca^{2+} transients • EC coupling • β-adrenergic response • action potentials	not determined	not determined



[1]Oh et al. Cardiac progenitor cells from adult myocardium : Homing, differentiation, and fusion after infarction. *Proc. Natl. Acad. Sci. (USA)*: **100**, 12313-12318 (2003)
[2]Martin et al. Persistent expression of the ATP-binding cassette transporter, Abcg2, identifies cardiac SP cells in the developing and adult heart. *Dev. Biol.*: **265**, 262-275 (2004)

Laugwitz, Nature 2005

Supplementary Table 2. Comparison of isl1$^+$ cardioblasts, cardiac sca-1$^+$ cells and cardiac side population (SP) cells.

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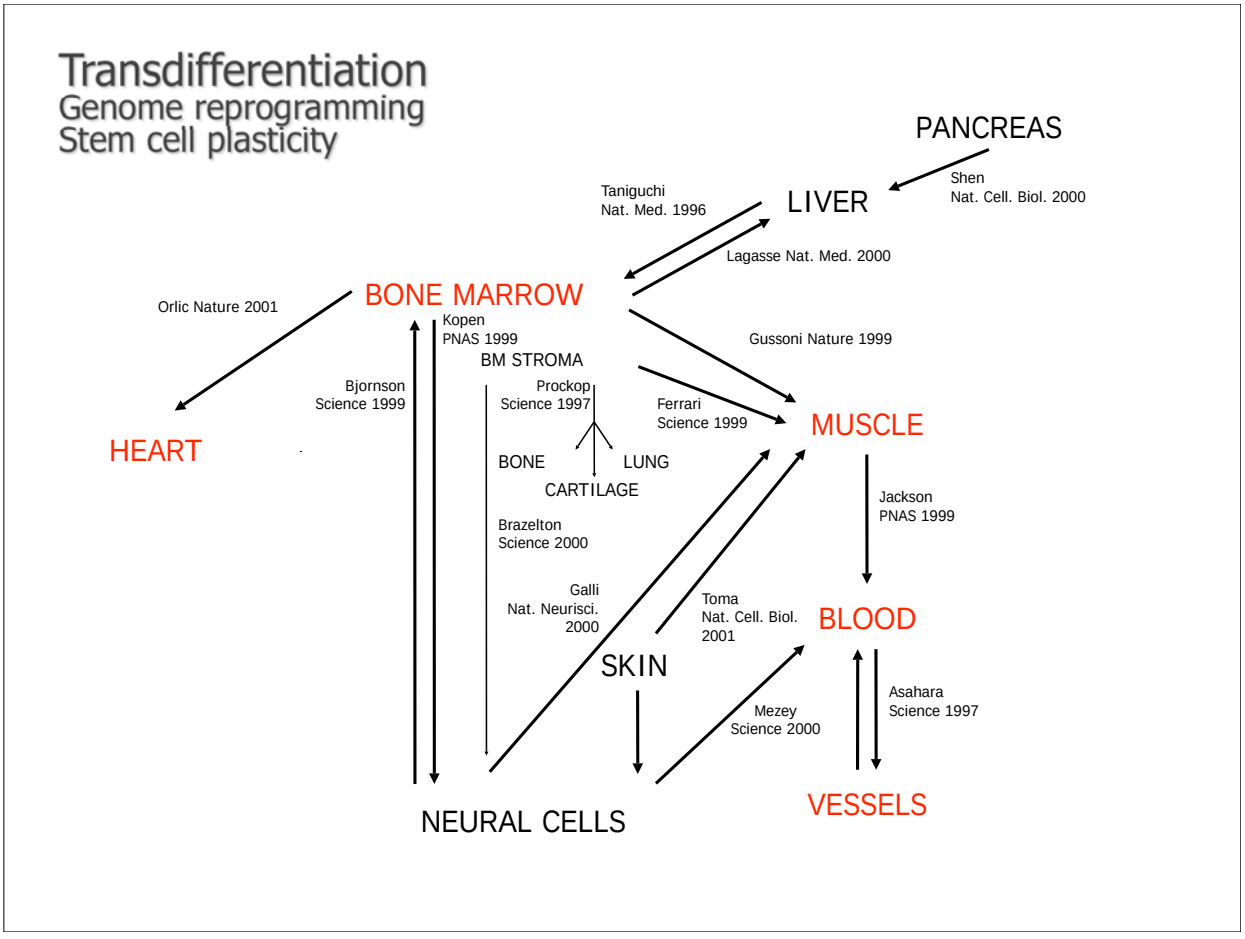
3. *in vivo* localization	• outflow tract • free wall of atria • intra-atrial septum • conus muscle • right ventricle	• adjacent to basal lamina • no preferred heart region	• not determined
4. Progenitor identity determined by lineage tracing	• isl1 identifies cardiac progenitor cells • established embryonic lineage marker for the heart	• sca-1 surface marker used for cell purification • no cardiac lineage marker	• Abcg2 activity used for Hoechst dye efflux • no cardiac lineage marker
5. Myocytic differentiation *in vitro*	α-actinin expression with sarcomeric structure : 22% cardiac troponin T : 25%	α-actin expression without sarcomeric structure : 4.6% cardiac troponin I : 2.8%	α-actinin expression without sarcomeric structure : % not determined
6. Myocytic differentiation *in vivo* after cell transplantation	not determined	ischemia/reperfusion injury: ~1.5% differentiation ~1.5% cell fusion	not determined
7. Functional evaluation of *in vitro* differentiated cells	• Ca^{2+} transients • EC coupling • β-adrenergic response • action potentials	not determined	not determined

[1]Oh et al. Cardiac progenitor cells from adult myocardium : Homing, differentiation, and fusion after infarction. *Proc. Natl. Acad. Sci. (USA)*: **100**, 12313-12318 (2003)
[2]Martin et al. Persistent expression of the ATP-binding cassette transporter, Abcg2, identifies cardiac SP cells in the developing and adult heart. *Dev. Biol.*: **265**, 262-275 (2004)



Turning Blood into Brain: Cells Bearing Neuronal Antigens Generated in Vivo from Bone Marrow

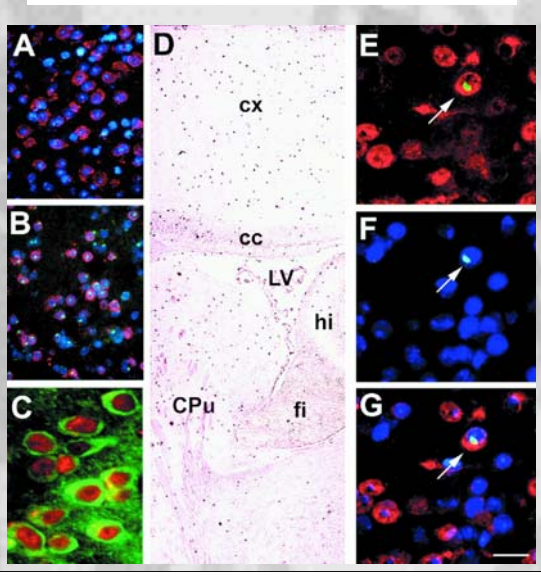
Éva Mezey,[1]* Karen J. Chandross,[2] Gyöngyi Harta,[1]
Richard A. Maki,[3,4] Scott R. McKercher[3]

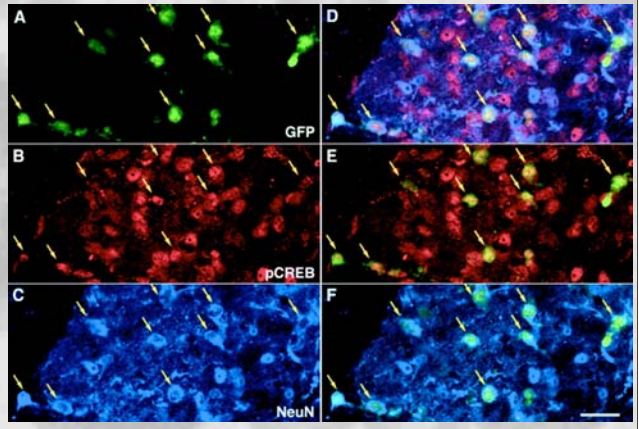
Bone marrow stem cells give rise to a variety of hematopoietic lineages and repopulate the blood throughout adult life. We show that, in a strain of mice incapable of developing cells of the myeloid and lymphoid lineages, transplanted adult bone marrow cells migrated into the brain and differentiated into cells that expressed neuron-specific antigens. These findings raise the possibility that bone marrow–derived cells may provide an alternative source of neurons in patients with neurodegenerative diseases or central nervous system injury.

From Marrow to Brain: Expression of Neuronal Phenotypes in Adult Mice

Timothy R. Brazelton, Fabio M. V. Rossi, Gilmor I. Keshet, Helen M. Blau*

After intravascular delivery of genetically marked adult mouse bone marrow into lethally irradiated normal adult hosts, donor-derived cells expressing neuronal proteins (neuronal phenotypes) developed in the central nervous system. Flow cytometry revealed a population of donor-derived cells in the brain with characteristics distinct from bone marrow. Confocal microscopy of individual cells showed that hundreds of marrow-derived cells in brain sections expressed gene products typical of neurons (NeuN, 200-kilodalton neurofilament, and class III β-tubulin) and were able to activate the transcription factor cAMP response element–binding protein (CREB). The generation of neuronal phenotypes in the adult brain 1 to 6 months after an adult bone marrow transplant demonstrates a remarkable plasticity of adult tissues with potential clinical applications.



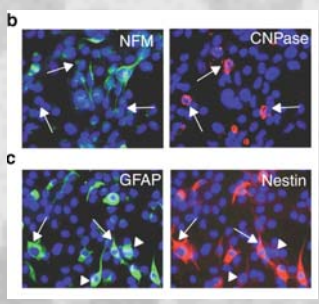


articles

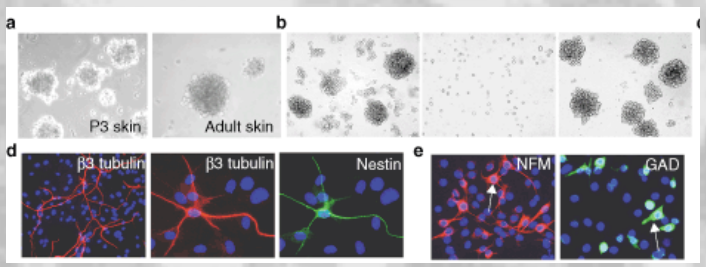
Isolation of multipotent adult stem cells from the dermis of mammalian skin

Jean G. Toma*, Mahnaz Akhavan*†, Karl J. L. Fernandes*†‡, Fanie Barnabé-Heider*, Abbas Sadikot§, David R. Kaplan*‡ and Freda D. Miller*¶

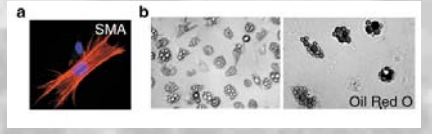
We describe here the isolation of stem cells from juvenile and adult rodent skin. These cells derive from the dermis, and clones of individual cells can proliferate and differentiate in culture to produce neurons, glia, smooth muscle cells and adipocytes. Similar precursors that produce neuron-specific proteins upon differentiation can be isolated from adult human scalp. Because these cells (termed SKPs for skin-derived precursors) generate both neural and mesodermal progeny, we propose that they represent a novel multipotent adult stem cell and suggest that skin may provide an accessible, autologous source of stem cells for transplantation.



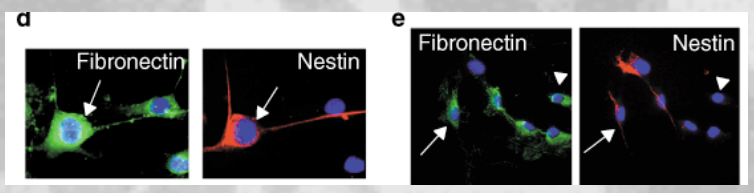
Glial cells generation



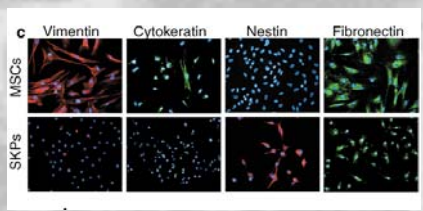
Neurogenic differentiation



Smooth muscle and adipocyte generation



Nestin (NSC marker) and fibronectin (MSC marker) expression

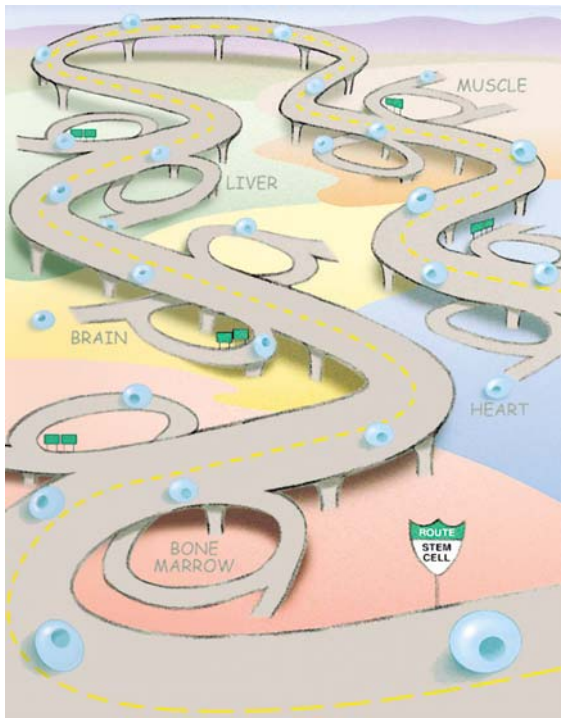


Comparison between SKP and MSC

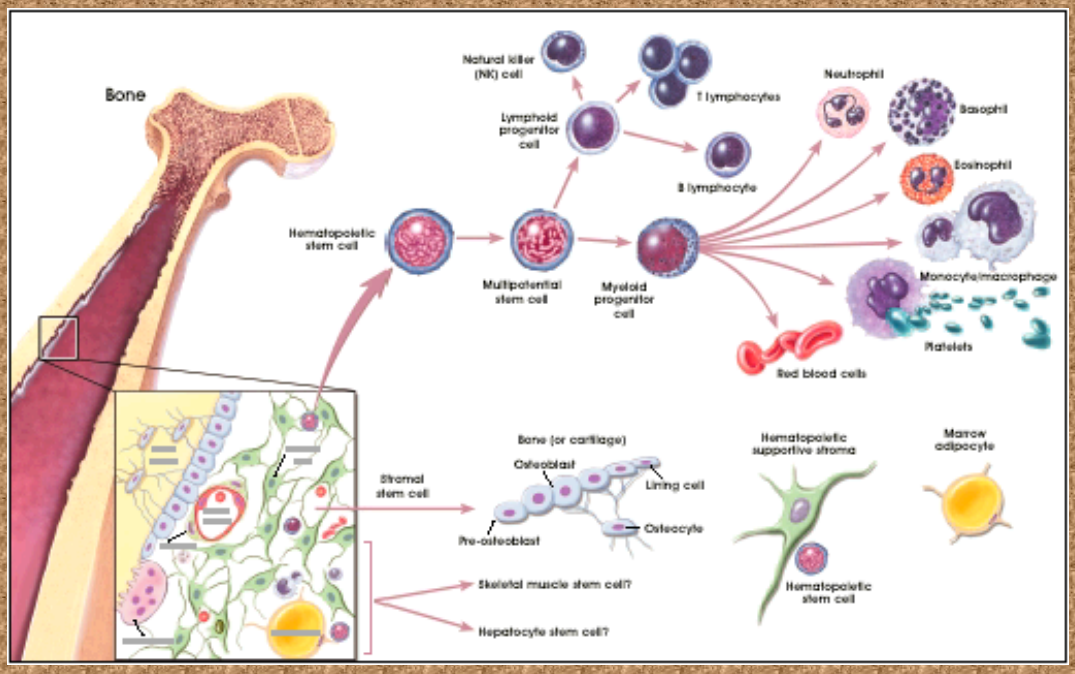
The evolving concept of a stem cell: entity or function?

"…rather then 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."

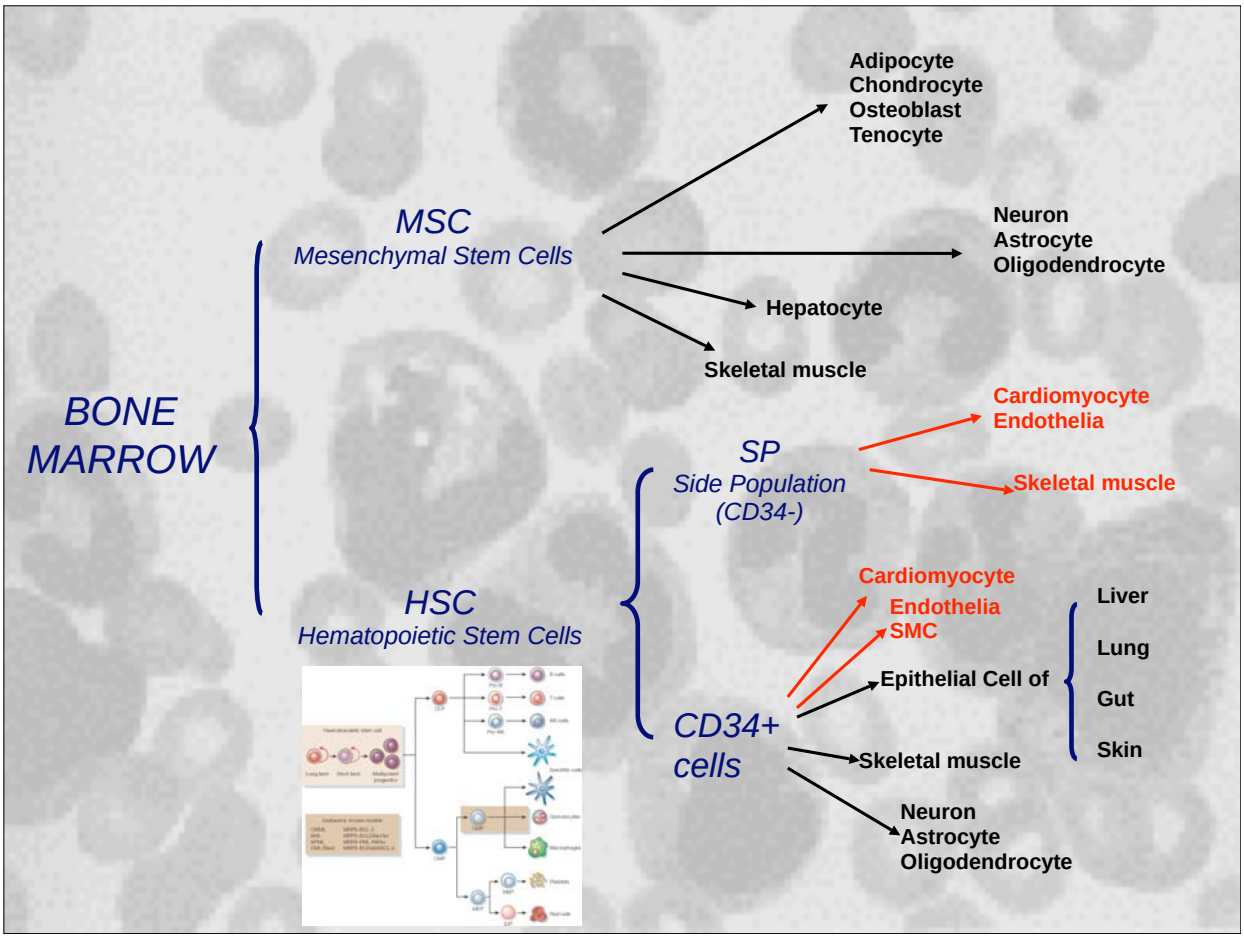
H. Blau. Cell, 2001



The bone marrow



- **BMT is a common clinical practice**
- **Established presence of stem cells in the bone marrow (HSCs and MSCs)**



Selected Cell-Therapy Trials

Coordinating center	Condition	Subjects	Status
University of Düsseldorf	heart attack	60	completed
University of Frankfurt	heart failure	200	ongoing
University Clinic, Hannover	heart attack	60	ongoing
Hôpital Européen Georges Pompidou	heart attack	300	ongoing
Seoul National University Hospital	heart attack	11	suspended
St. Elizabeth's Medical Center, Boston	blocked arteries	24	ongoing
BioHeart Inc., Weston, Florida	heart failure	15	ongoing
Texas Heart Institute, Houston	blocked arteries/ heart failure	30	ongoing

9 APRIL 2004 VOL 304 SCIENCE

Neovascularization of ischemic myocardium by human bone-marrow–derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function

A.A. KOCHER[1], M.D. SCHUSTER[1], M.J. SZABOLCS[3], S. TAKUMA[2], D. BURKHOFF[2], J. WANG[1], S. HOMMA[2], N.M. EDWARDS[1] & S. ITESCU[1,2]

Kocher AA., Nature Medicine, Apr. 2001

Bone marrow cells regenerate infarcted myocardium

Donald Orlic†, Jan Kajstura*, Stefano Chimenti*, Igor Jakoniuk*, Stacie M. Anderson†, Baosheng Li*, James Pickel‡, Ronald McKay‡, Bernardo Nadal-Ginard*, David M. Bodine†, Annarosa Leri* & Piero Anversa*

NATURE | VOL 410 | 5 APRIL 2001

Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells

Kathyjo A. Jackson,[1] Susan M. Majka,[1,2,3] Hongyu Wang,[1] Jennifer Pocius,[4] Craig J. Hartley,[4] Mark W. Majesky,[3,5] Mark L. Entman,[4] Lloyd H. Michael,[4] Karen K. Hirschi,[1,2,3] and Margaret A. Goodell[1]

The Journal of Clinical Investigation | June 2001 | Volume 107 | Number 11

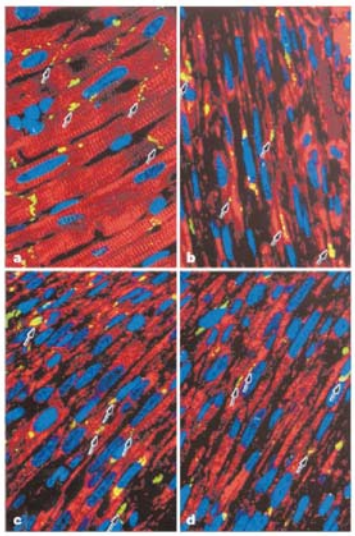


Figure 4 Myocardial repair and connexin 43. **a**, Border zone; **b**–**d**, regenerating myocardium. Shown are connexin 43 (yellow–green; arrows indicate contacts between myocytes) and α-sarcomeric actin (red), and PI-stained nuclei (blue). Original magnification, ×500 (**a**), ×800 (**b**–**d**).

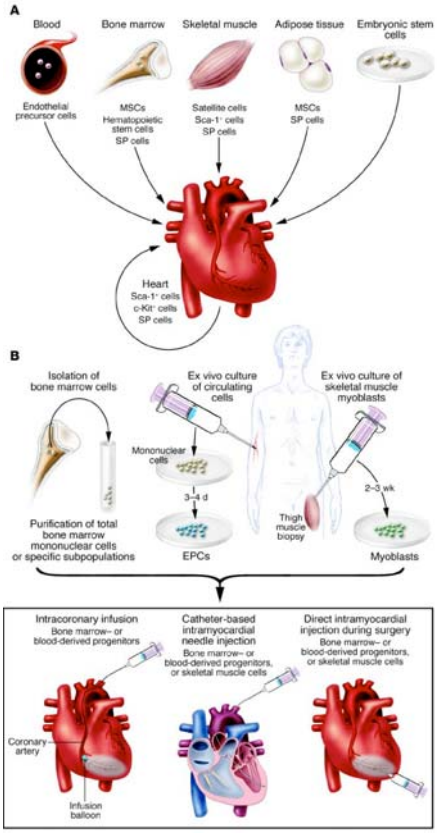
Source of stem cells for potential heart injection

BM mononuclear cells
EPCs (CD133+ CD34+ VEGR2+)
Culture-expanded myelomonocytic EPCs (CD14+ CD34-)
Mesenchymal Stem Cells (CD34- CD133-)
Skeletal myoblasts
Resident Cardiac Stem Cells
Embryonic Stem Cells

Modes of cell delivery

Transvascular
- Intracoronary (stop-flow balloon cathether)
- Intravenous
- After progenitor cell mobilization

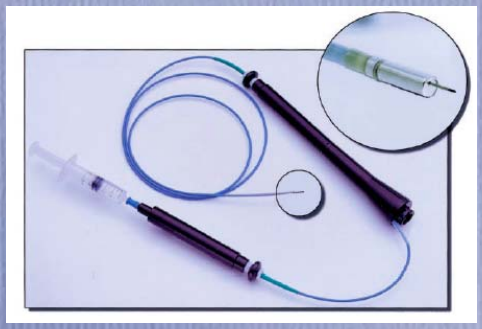
Direct injection in the ventricular wall
- Transendocardial injection
- Transepicardial injection (during CABG)
- Transcoronary vein injection



The Journal of Clinical Investigation
Volume 115 Number 3 March 2005

The NOGA system for transmyocardial injection

An injection catheter is incorporates the mapping capabilities of the system. This provide a means by which tissues with different degrees of viability and ischemia can be mapped in detail, allowing therapy to be precisely targeted (eg, at the border zone of an infarct)



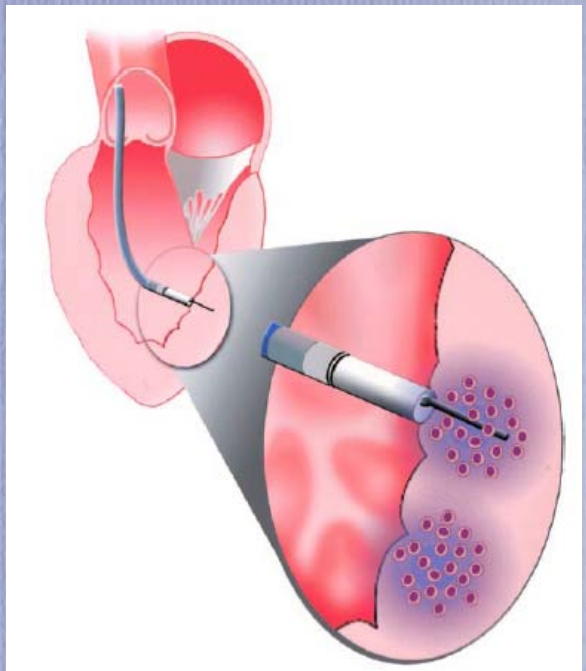
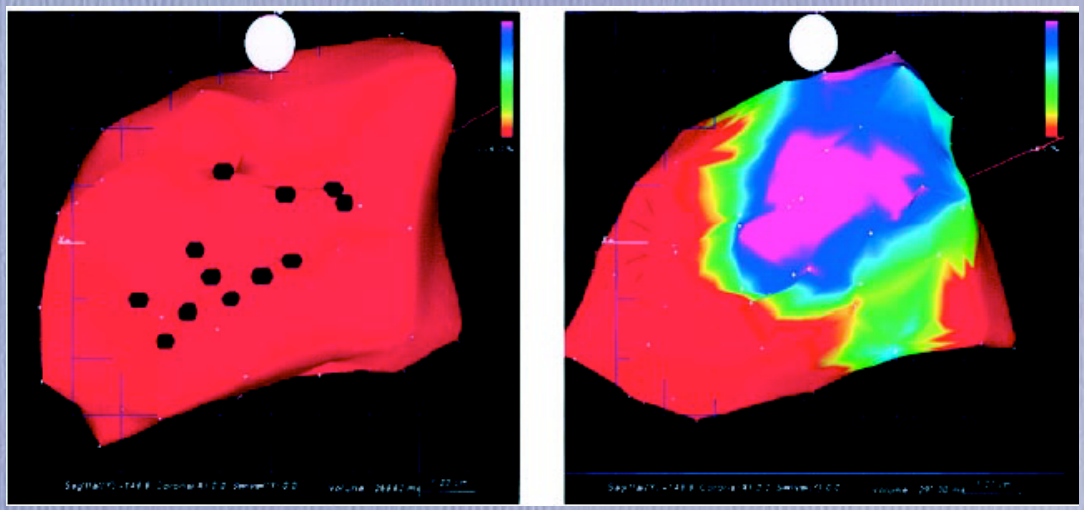


Figure 2. Injection catheter advanced into the left ventricle through the aortic valve. The catheter tip is placed against the endocardial surface (insert) with the needle extended into the myocardium delivering ABMMNCs.



Left, electromechanical linear local shortening map from a stem cell injection procedure. The red color represents low contractility (severe cardiomyopathy). The black dots are injection sites.

Right, similar map at 4 month follow-up, showing dramatic improvement in contractility at the site of prior cell injection.

Stem cells

Lost in translation

Kenneth R. Chien

The potential use of stem cells as agents of repair in human disease makes them the subject of high-profile studies. But we should be wary of prematurely pushing laboratory research into clinical practice.

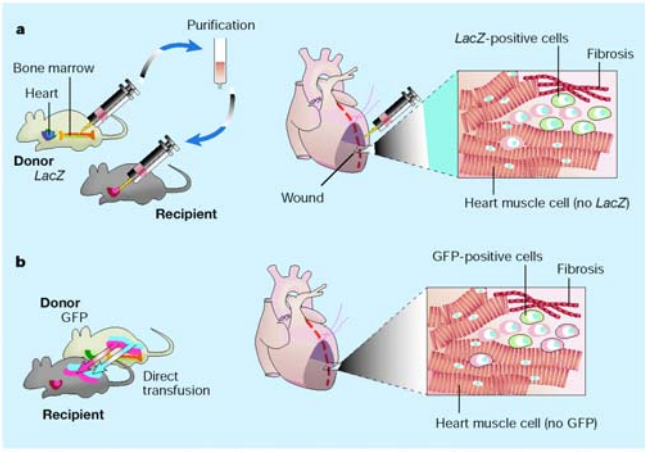


Figure 1 **Two strategies used to show that bone-marrow stem cells do not take on the role of damaged heart cells.** a, **Murry *et al.*[2] isolated and purified genetically modified bone-marrow stem cells from mice. The modification 'tagged' the cells (with *LacZ*), enabling them to be detected in the recipient mouse heart, into which the cells were directly injected. Closer inspection of the recipient heart showed that the label could not be detected in heart muscle cells.** b, **Similar results were shown by Balsam *et al.*[3], although the approach was slightly different. Donor bone-marrow stem cells were transfused directly into the circulation of recipients. Again, the tag (GFP; green fluorescent protein) could not be detected in heart muscle cells of the donor; indeed, the bone-marrow cells continued to differentiate into blood cells while in the heart.**

Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium

Leora B. Balsam[1], Amy J. Wagers[2,3], Julie L. Christensen[2,3], Theo Kofidis[1], Irving L. Weissman[2,3] & Robert C. Robbins[1]

[1]*Departments of Cardiothoracic Surgery,* [2]*Pathology, and* [3]*Developmental Biology, Stanford University School of Medicine, Stanford, California 94305, USA*

Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts

Charles E. Murry[1], Mark H. Soonpaa[2], Hans Reinecke[1], Hidehiro Nakajima[2], Hisako O. Nakajima[2], Michael Rubart[2], Kishore B. S. Pasumarthi[2]*, Jitka Ismail Virag[1], Stephen H. Bartelmez[3], Veronica Poppa[1], Gillian Bradford[2], Joshua D. Dowell[2], David A. Williams[2]* & Loren J. Field[2]

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Cardiac Cell Therapy — Mixed Results from Mixed Cells

Anthony Rosenzweig, M.D.

N ENGL J MED 355;12

Table 1. Randomized, Controlled Trials of BMC for Cardiac Disease.*

Trial or Investigator Group	Setting	Design	No. of Cells Administered in Treatment Group	Results
BOOST[4,9]	PCI after acute myocardial infarction	Randomized trial 30 patients received BMC; 30 received no infusion LVEF assessed by MRI	Approximately 2.5×10^9 unfractionated BMC	At 6 mo: LVEF 6% greater in BMC group than in control group At 18 mo: no significant difference in LVEF between the 2 groups
Janssens et al.[8]	PCI after acute myocardial infarction	Randomized, double-blind trial 33 patients received BMC; 34 received placebo infusion LVEF was assessed by MRI	Approximately 3×10^8 Ficoll-separated BMC	At 4 mo: no significant difference in overall LVEF; decreased infarct size and better regional function in BMC group
TOPCARE-CHD[6]	Chronic left ventricular dysfunction	Randomized, crossover trial In the second phase, 24 patients received CPC, 28 received BMC, 23 received no infusion LVEF assessed by left ventricular angiography	Approximately 2×10^8 Ficoll-separated BMC or approximately 2×10^7 Ficoll-separated, cultured CPC	At 3 mo: greater increase in LVEF (2.9 percentage points) in BMC group than in CPC group or control group
ASTAMI[7]	PCI after acute myocardial infarction	Randomized trial 47 patients received BMC; 50 received no infusion LVEF assessed by SPECT, echocardiography, and MRI	Approximately 7×10^7 Ficoll-separated BMC	At 6 mo: no significant difference in LVEF between the 2 groups
REPAIR-AMI[5]	PCI after acute myocardial infarction	Randomized, double-blind trial 101 patients received BMC; 98 received placebo infusion LVEF assessed by left ventricular angiography	Approximately 2.4×10^8 Ficoll-separated BMC	At 4 mo: greater absolute increase in LVEF in BMC group than in placebo group (5.5% vs. 3.0%) At 1 yr: reduction in combined adverse clinical events in BMC group as compared with placebo group

* BOOST denotes Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration, PCI percutaneous coronary intervention, MRI magnetic resonance imaging, TOPCARE-CHD Transplantation of Progenitor Cells and Recovery of LV Function in Patients with Chronic Ischemic Heart Disease, CPC progenitor cells derived from circulating blood, ASTAMI Autologous Stem-Cell Transplantation in Acute Myocardial Infarction, SPECT single-photon-emission computed tomography, and REPAIR-AMI Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction.

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