

PRINCIPE FELIPE **CENTRO DE INVESTIGACION**





Fondo Europeo de Desarrollo Regional









PRINCIPE FELIPE CENTRO DE INVESTIGACION

PRESENT AND FUTURE PERSPECTIVES OF **REGENERATIVE MEDICINE**

Valencia, 24 September 2008



Scientists have developed a chicken withOnewing and three legs!













Louise Brown just after birth (1978)

Louise just after her birth, with Patrick Steptoe (right) and Robert Edwards







Louise Brown in 2003, with mum Lesley and dad John



Phylogenetic Tree of Life













President Bush Discusses Stem Cell Veto and Executive Order "Expanding Approved Stem Cell Lines in Ethically Responsible Ways"

President George W. Bush embraces stem cell patient Kaitlyne McNamara following his address on the reasons he vetoed S.5, the "Stem Cell Research Enhancement Act of 2007," in the East Room of the White House Wednesday, June 20, 2007. McNamara was born with spina bifida, a disease that damaged her bladder, her doctors isolated healthy stem cells in a piece of her own bladder and used them to grow her a new bladder.

the "Human **Embryonic** Stem Cell Registry" the "Human **Pluripotent** Stem Cell Registry"

White House photo by Eric Draper













"You're a selfish bastard, Lewis...! Those stem cell lines were meant for people who've LOST an organ!"



STEM CELLS









STEM CELLS







Cell lines

Attached

Blastocyst cultured on feeder







- Unspecialized (undifferentiated)
- Capable of long-term self renewal (dividing and renewing themselves for long periods)
 - It took 20 ys to learn how to grow hESC following the development of conditions for growing mouse ESC
- Can give rise to specialized cells (differentiation)
 - Internal signals controlled by cell's genes
 - External signals (cell contact, molecules in microenviroment)





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Characteristic surface and intracellular markers of hESC

	TRA-1-60, TRA-1-81, alkaline
Surface markers	phosphatase, GTCM-2
Transcription factors	Oct-4, nanog, Sox2, Rex1
Other markers	Telomerase activity
Karyotype	Normal, 46, XX or XY
Doubling time in culture	24–48 h

Rex1, zinc finger protein 42; SOX2, SRY-related HMG box gene 2; TRA and GTCM are antibodies that recognize specific epitopes on proteoglycans or associated proteins on the cells surface.

The expression of stage-specific embryonic antigens (SSEAs) can be used to identify whether ESCs are undifferentiated or have started to mature. The SSEAs on hESC differ from those on murine ESC; undifferentiated hESCs are positive for SSEA-3 and -4 but lack SSEA-1, whereas murine ESCs have the opposite expression pattern.



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Self renewal

 when ESC divide one daughter cell will be an identical copy of the parent and will remain a stem cell, the other daughter cell may also be identical (symmetrical division) or may go on to differentiate (asymmetrical division)





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STEM CELLS

EMBRYONIC

- Pluripotent (all cell types of the body)
- Large number easily grown
 in culture
- Potential rejection

ADULT

- Multipotent (cell types of the tissue), plasticity
- Rare in mature tissues and expansion in culture unknown
- No rejection (autologous)











POTENTIAL USES OF HUMAN STEM CELLS

- hESC may yield information about human development (how undifferentiated SC become differentiated)
 - Cancer and birth defects are due to abnormal cell division and differentiation
- hESC could be used to test new drugs
- Cell-based therapies
 - Parkinson's disease
 - Spinal cord injury
 - Stroke
 - Burns
 - Heart disease
 - Diabetes
 - Osteoarthritis , and rheumatoid arthritis



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SCIENCE (2006) 311: 632



NEW NEURONS FOLLOW THE FLOW OF CEREBROSPINAL FLUID IN THE ADULT BRAIN

Kazunobu Sawamoto, Hynek Wichterle, Oscar Gonzalez Perez, Jeremy A. Cholfin, Masayuki Yamada, Nathalie Spassky, Noel S. Murcia, **Jose Manuel Garcia-Verdugo**, Oscar Marin, John L. R. Rubenstein, Marc Tessier-Lavigne, Hideyuki Okano, Arturo Alvarez-Buylla





Frogs (Xenopus tropicalis)



Medaka fish (Oryzias latipes)











STEM CELL DIFFERENTIATION BIOLOGY



TRIDIMENSIONAL IMAGE OF AXONAL CONECTION BETWEEN OLFATIVE GLOMERULI AND BRAIN, AND EYES AND OPTICAL TECTUM OF MEDAKA FISH STAINED WITH ACETYLATED TUBULIN
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Supply of specialized cells







and

f

- COOH



SCREENING OF REGULATORY AREAS TO CHANGE IN VIVO FUNCTION



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EMBRYONIC STEM CELLS

Lineage-specific differentiation of hESC to clinically relevant cell types

Tissue type	Reference	Cell lineage	Disease application
		Dopamine	
Neural	<u>Zeng et al. (2004)</u>	Ottigrootendrocyt	Parkinson's disease
	<u>Nistor et al. (2005)</u>	е	Spinal cord injury
	<u>Li et al. (2005)</u>	Motor neuron	Motor neuron disease, spinal cord injury
Liver	<u>Hay et al. (2007)</u>	Hepatocyte	Liver failure
Endocrine	<u>D'Amour et al. (2006)</u>	Islet β-cells	Diabetes
Cardiovascular	Mummery et al. (2002)	Cardiomyocyte	Myocardial infarction
			Stroke, myocardial infarction, ischaemic
	<u>Wang et al. (2007)</u>	Endothelial	limb disease
		Retinal	
Eye	<u>Haruta (2005)</u>	epithelium	Retinal degeneration or injury
Skin	<u>Ji et al. (2006)</u>	Keratinocytes	Burns, traumatic skin loss
Musculoskeletal	<u>Zheng et al. (2006)</u>	Skeletal muscle	Muscular dystrophy
	<u>Toh et al. (2007)</u>	Chondrocytes	Cartilage loss
			Loss from trauma or tumour, brittle bone
	<u>Karner et al. (2007)</u>	Bone	disease
Blood	Vodyanik et al. (2006)	Lymphoid cells	Immune deficiencies
	<u>Olivier et al. (2006)</u>	Erythrocytes	Blood loss, sickle cell anaemia

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ENFERMEDAD DE PARKINSON



Coordenadas entre **P1** (Sustancia Negra) y **P2** (Estriado), y los ángulos (a= 28.20° y b= 27.47°). **nv** representa la distancia entre P1 y P2 (nv= 6.138 mm). (a) anterior; (**p**) posterior; (**m**) medial; (**I**) lateral; (**d**) dorsal y (**v**) ventral.

The Rat Brain in Stereotaxic Coordinates" (Paxinos G. and Watson C., 1997)



ENFERMEDAD DE PARKINSON



Inmunotinción frente a tirosina hidroxilasa (TH): (**A**) sección coronal de cerebro de rata de 20µm contrastada con azul de toluidina, de un animal lesionado mediante axotomía en la que se observa la denervación del estriado; (**B**) sección coronal de cerebro de rata de un animal lesionado químicamente con 6-OHDA, donde se observa la denervación del estriado derecho; (**C**) total eliminación de células dopaminérgicas de la sustancia negra del hemisferio derecho en el modelo de 6OHDA.



PARKINSON'S DISEASE





ENFERMEDAD DE PARKINSON



Sistema de implante de nervio periférico (cánula y fiador)



ENFERMEDAD DE PARKINSON Localización del nervio



Nervio ciático en secciones coronales de cerebro 4 semanas después del implante (azul de toluidina)



Inmuno tinción frente a tirosina hidroxilasa (TH). (**A**) La línea de puntos delimita el nervio. (**B**) Magnificación en la que se observa la presencia de fibras THinmunoreactivas en el interior del nervio ciático. (**SNpc**) Sustancia Negra pars compacta, (**SNpr**) Sustancia Negra pars reticular.



PARKINSON'S DISEASE Biomaterials



Ejemplos de estructuras porosas utilizadas como scaffolds, producidas en materiales poliméricos bioestables y biodegradables





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The Journal of Neuroscience, 26(24):6627-6636 (2006)





SUBVENTRICULAR ZONE-DERIVED NEUROBLASTS MIGRATE AND DIFFERENTIATE INTO MATURE NEURONS IN THE POST-STROKE ADULT STRIATUM

Toru Yamashita, Mikiko Ninomiya, Pilar Hernández Acosta, **Jose Manuel García-Verdugo**, Takehiko Sunabori, Masanori Sakaguchi, Kazuhide Adachi, Takuro Kojima, Yuki Hirota, Takeshi Kawase, Nobuo Araki, Koji Abe, Hideyuki Okano, and Kazunobu Sawamoto

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CARDIOMYOCYTES AND ADULT MESENCHYMAL STEM CELLS







CARDIOMYOCYTES FROM HUMAN EMBRYONIC STEM CELLS





TOLUIDINE BLUE STAINING



TROPONIN



RT-PCR (CARDIAC ACTIN EXPRESSION)









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ALTERATION OF NORMAL ARCHITECTURE IN SPINAL CORD INJURY



HESC DIFFERENTIATION IN MOTONEURONS AND OLIGODENDROCYTES



MOTONEURON PRECURSORS

OLIGODENDROCYTE PROGENITORS



RAT SPINAL CORD EPENDYMAL CELL DIFFERENTIATION



EMBRYOID BODIES



OLIGODENDROCYTE PROGENITORS







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DIABETES WORLDWIDE PREVALENCE

In 1985 the number of cases with type 2 diabetes worldwide was 30 million, the more recent number is 150 million, and in the next 20 or so years this disease is expected to double in prevalence to 300m



Diabetes in Spain: 6% of population 3rd cause of death among women; 7nd among men



EMBRYONIC STEM CELLS

Schematic representation of the four-stage differentiation protocol from ES cells (ES) through mesendoderm (ME) to definitive endoderm (DE), primitive gut tube (PG), posterior foregut (PF) and finally pancreatic endoderm (PE), with applied factors and media shown below

Stage 1		Stage 2	Stage 3	Stage 4	
	Definitive endoderm		Primitive gut tube	Posterior foregut	Pancreatic endoderm and endocrine precursors
	ActA + Wnt	ActA	KGF	RA + Cyc + Nog	No factors
	RPMI + 0% FBS	RPMI + 0.2% FBS	RPMI + 2% FBS	DMEM + 1% B27	DMEM + 1% B27
	1 day	2 days	3 days	3 days	3 days
E	s N		DE F	PG F	PF P
	XT4 B NOG F X2 W AD N	RA S GF4 C VNT3 F ICAD C	OX17 HN ER HN OXA2 XCR4	NF1B PE NF4A HN PF SC	DX1NKNF6PTIROX1NGDX9NK

The identification of precursor populations for many tissues has also led to the development of multistep culture protocols for the derivation of individual lineages. For example, it was found that the most efficient method of generating cells that can produce insulin and resemble pancreatic beta cells was via a number of intermediate stages induced by altering culture conditions. The hESC passed through mesendoderm, definitive endoderm, primitive gut tube, posterior foregut and pancreatic endoderm stages before finally generating hormone-expressing endocrine cells. Such culture systems and the identification of these populations and their potential for further differentiation are not only based on knowledge from developmental biology but also rely on an element of empirical input based on the knowledge of the scientist working with these cells.





Transplantation of embryonic stem cell-derived insulin-positive cells into diabetic mice left renal capsule ameliorates the diabetic symptom



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THE MOST COMMON CAUSES OF BLINDNESS AROUND THE WORLD

- cataracts (47.8%),
- glaucoma (12.3%),
- uveitis (10.2%),
- age-related macular degeneration (AMD) (8.7%),
- trachoma (3.6%),
- corneal opacity (5.1%), and
- diabetic retinopathy (4.8%)
- other causes

WHO , 2002





AMD - Age Related Macular Degeneration

- Most common cause of blindness in Spain in patients over 55 years of age
- 525,000 patients significantly visually impaired in Spain and 8 million worldwide
- Increasing prevalence with age (rare under 60yrs peak incidence >80yrs)
- 26,000 new cases every year in Spain
- 3 million Spaniards who are at risk of suffering from DMAE



Amsler grid



Dry AMD



Wet AMD

Dry AMD is the most common form (85%). It develops very slowly causing gradual loss of central vision. No medical treatment for this type. <u>Wet AMD (Advanced)</u> accounts for 15% of AMD cases. Subretinal neovascularisation from choroidal blood vessels causes bleeding and scarring. Can develop quickly and leads to significantly more vision loss. Dysfunction of the retinal pigment epithelium (RPE)/Bruch's membrane-choriocapillaris complex initiates the degeneration of macular photoreceptors which causes central visual loss



Macula - The central zone of the retina where the most critical vision is enabled








Age Related Macular Degeneration (AMD)



Normal vision

Viewed by person with AMD

Differentiation of hESC for treatment of retinal disorders

- One of the cell types that has been shown to integrate successfully into host retina and restore vision are retinal cells that were already committed towards a photoreceptor fate.
- An equivalent donor cell in the human would have to be obtained from the 2nd trimester which raises ethical concerns.
- A very attractive source of investigation is to try and differentiate human embryonic stem cells (hESC) into photoreceptors in vitro prior to transplantation.
- The aim is to enhance and optimise the directed differentiation of hESCs using our multi-step protocol and test their engraftment in animal models of retinal disease





hESC colony

Cone photoreceptors



Ciliary body

There are approximately 6 million cones in our retina, and they are sensitive to a wide range of brightness. The three different types of cones are sensitive to short, medium and long wavelengths, respectively.

Additionally, we have approximately 125 million rods on the retina, which are used only in dim light, and are monochromatic – black and white.)







^a|There are six classes of neuron in the mammalian retina: rods (1), cones (2), horizontal cells (3), bipolar cells (4), amacrine cells (5) and retinal ganglion cells (RGCs) (6).

They have a laminar distribution (OS/IS, outer and inner segments of rods and cones; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; NFL, optic nerve fibre layer). b | A cone pedicle, the synaptic terminal of cones. Four presynaptic ribbons are apposed to the invaginating dendrites of horizontal cells (yellow) and ON cone bipolar cells (blue). This synaptic arrangement is called a 'triad'. OFF cone bipolar cell dendrites form contacts at the cone pedicle base (purple). c | A rod spherule, the synaptic terminal of rods. The presynaptic ribbon is apposed to the invaginating axons of horizontal cells (yellow) and the dendrites of rod bipolar cells (blue). OFF cone bipolar cell dendrites form contacts at the base (purple). d | The axon terminal of a cone bipolar cell (blue) contains up to 50 presynaptic ribbons, and connects to postsynaptic amacrine cell processes (orange) and RGC dendrites (purple). e | A magnified view of a bipolar cell ribbon synapse (blue) with an amacrine cell process (orange) and an RGC dendrite (purple). The amacrine cell provides a feedback synapse onto the bipolar cell. This synaptic arrangement is called a 'dyad'. Amacrine cells also form numerous conventional synapses throughout the IPL with RGCs and other amacrine cells (not shown). Some amacrine cell processes reach out to the OPL and there they provide synaptic contacts (interplexiform processes).





Protocol for hESCs differentiation



- survival, incorporation and differentiation of hESC-derived photoreceptor cells following transplantation into the eyes of *Crx-/-* mice
- visual response of Crx-/- and control mice transplanted with hESC-derived photoreceptor cells to light



- · Crx-/- lack photoreceptor outer segments and as result they lack rod and cone activity
- All retinal layers appear intact at birth. Photoreceptors start degenerating at one month postnatal and by 6 months the retina completely lacks outer nuclear and plexiform layers, resulting in tissue that is much thinner than normal.
- The inner retina remains largely intact.

STEPS FOR SUCCESSFUL CELL-BASED TREATMENTS

Stem Cells must:

- Proliferate extensively and generate sufficient quantities of tissue
- Differentiate into the desired cell type(s)
- Survive in the recipient after transplant
- Integrate into the surrounding tissue after transplant
- Function appropriately for the duration of the recipient's life
- Avoid harming the recipient in any way
- Avoid immune rejection

Enucleación











Dr. Andrew H. Crosby Section of Medical Genetics Division of Clinical Developmental Sciences St George's University of London, London

Main focus:

- Identification of genes and subsequent exploration of pathogenic mechanisms responsible for neurological disease, with a particular focus on neurodegenerative diseases of the motor neuron.
- Inherited epilepsy syndromes.

Hereditary Spastic Paraplegia

Motor neuropathy; SPG17/dHMNV/CMT Spastic paraplegia; SPG20 Spastic paraplegia with dementia; SPG21

Inherited Epilepsy Syndromes

autosomal recessive infantile-onset symptomatic epilepsy syndrome/ GM3 deficiency; SIAT9 mutation





(Nat Genet. 36{3}:271-6, 2004) Nat Genet. 31{4}:347-8, 2002). (Am. J. Hum Genet. 73{6}:1147-56, 2003)

(Nat. Genet.36{11}:1225-9, 2004)



Hereditary Spastic Paraplegia

<u>Synonyms</u>

Familial Spastic Paraplegia FSP HSP Spastic Spinal Familial Paralysis Strumpell's Familial Paraplegia Strumpell-Lorrain Familial Spasmodic Paraplegia Strumpell Disease Strumpell-Lorraine Syndrome

➢ Group of inherited neurological disorders characterized by progressive weakness (<u>paraplegia</u>) and increased muscle tone and stiffness (<u>spasticity</u>) of leg muscles.

➤ 1 in 20.000 individuals in the general population of Europe, with variable frequencies in distinct populations (from 1.3 to 9/100.000 individuals)

Symptom onset may occur as early as infancy or as late as the eighth or ninth decade of life; however, symptoms may most often develop during <u>early to mid-adulthood</u>







Hereditary Spastic Paraplegia

Disorder Subdivisions

- Hereditary spastic paraplegia, uncomplicated ("pure")
- Hereditary spastic paraplegia, complicated
 - + visual (optic neuropathy, retinopathy)
 - + hearing impairment (deafness),
 - + mental retardation
 - + dementia
 - + impaired control of voluntary movements (ataxia),
 - + icthyosis (dry, rough, scaly skin),
 - + peripheral neuropathy



• The basic underlying defect or defects in HSP are unknown, but associated symptoms appear to result from <u>progressive</u> <u>degenerative</u> changes of regions of the spinal cord (<u>corticospinal tracts</u>) that convey motor impulses from the brain to muscles involved in controlling certain voluntary movements









Stojkovic et al., RBM Online







IPS CELLS





INDUCTION OF STEM CELLS FROM ADULT CELL CULTURES BY OTHER DEFINED FACTORS



Microphotographs of plates and cells treated with a novel factor. White arrow localise single colony in the control group. Inserts represent high magnification of typical colonies and cells formed *in vitro* after treatment







GMP FACILITIES

















G











SELECTIVE BIOHYBRIDS

























ANALYSIS OF ADULT PANCREAS Macroscopic Analysis of ESC Derived Pancreas







Scientists have developed a chicken with**one**wing and three legs!







Malcolm Logan, Clifford J. Tabin Department of Genetics, Harvard Medical School

















"After 3.8 billion years of evolution, nature has learned - what works, what is appropriate and what lasts"

Randolph Bengas (1817-1848)

Founding principle is to seek out what Mother Nature has perfected over millions of years and mimic that engineering. This is known as **biomimicry**.









Holt-Oram Syndrome (Tbx5 gene defect)



Amputation of body extremity by trauma or surgery


















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