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CENTRO ANDALUZ DE BIOLOGÍA MOLECULAR & MEDICINA REGENERATIVA

Medicina Regenerativa y Pediatría
Pasado, Presente y Futuro

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Miguel Marín

VISSUM
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Medicina Regenerativa y Pediatría:

¿Sinónimos?

- *Stem Cells and Regenerative Medicine*
- *Cellular Medicaments: a Change in the Paradigm*
- *Cell Therapy in Pediatric Diseases*

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- *Stem Cells and Regenerative Medicine*

- *Cellular Medicaments: a Change in the Paradigm*
- *Cell Therapy of Liver Diseases*

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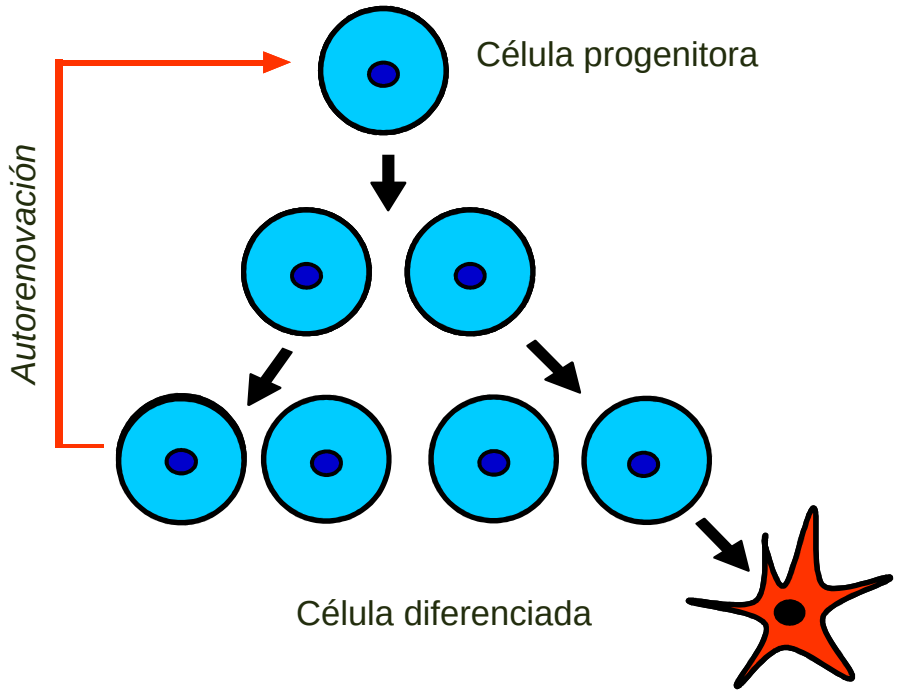
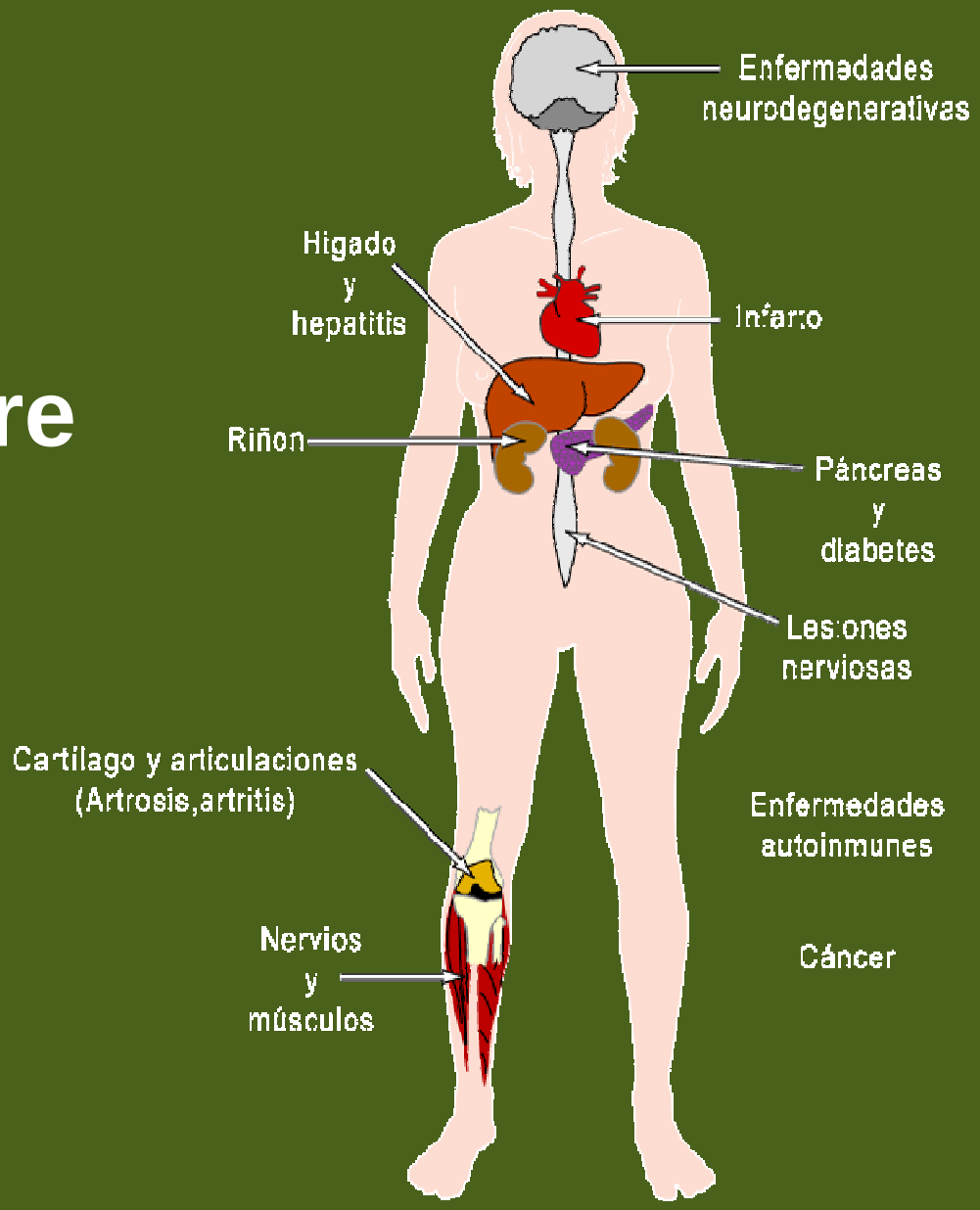
Stem Cell Concept

Células Madre (Troncales)

a. *Autorenovación*
b. *Diferenciación hacia otros tipos celulares*
c. *Capacidad para colonizar y regenerar un tejido*



Utilidad de las Células Madre



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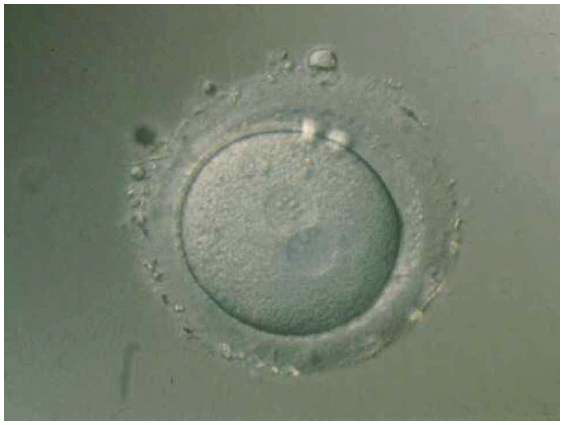
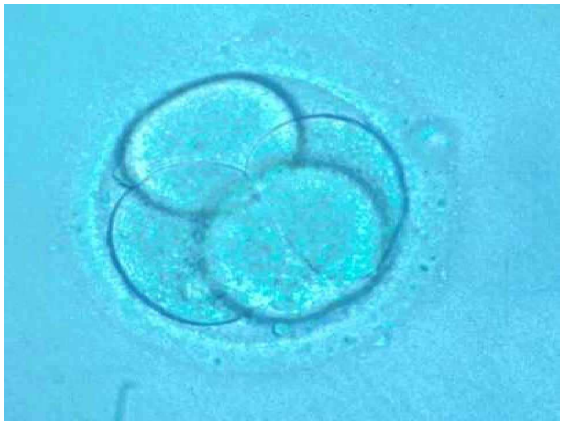
“Células ESC Humanas”



Día 0



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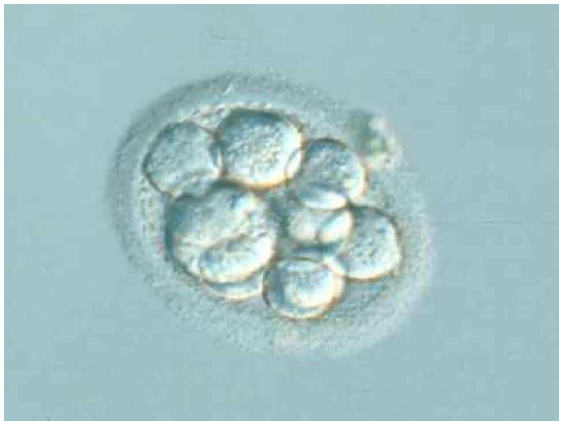

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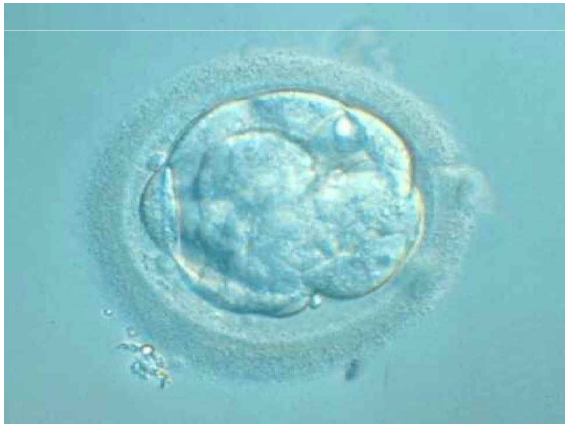

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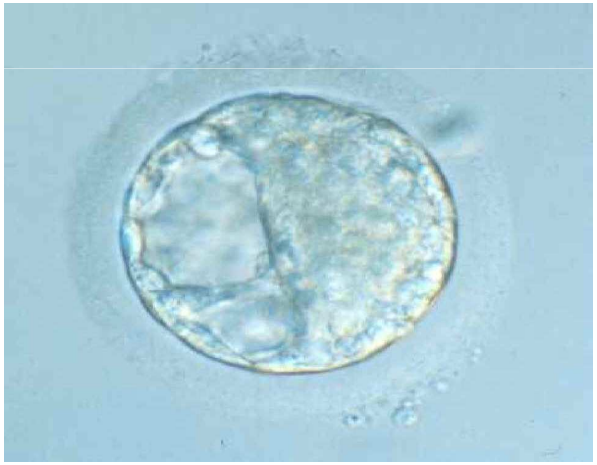
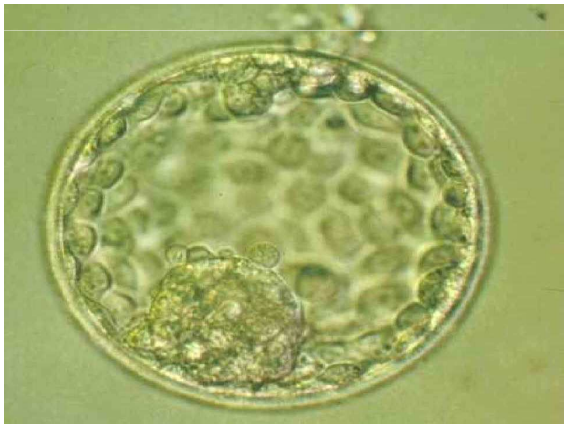


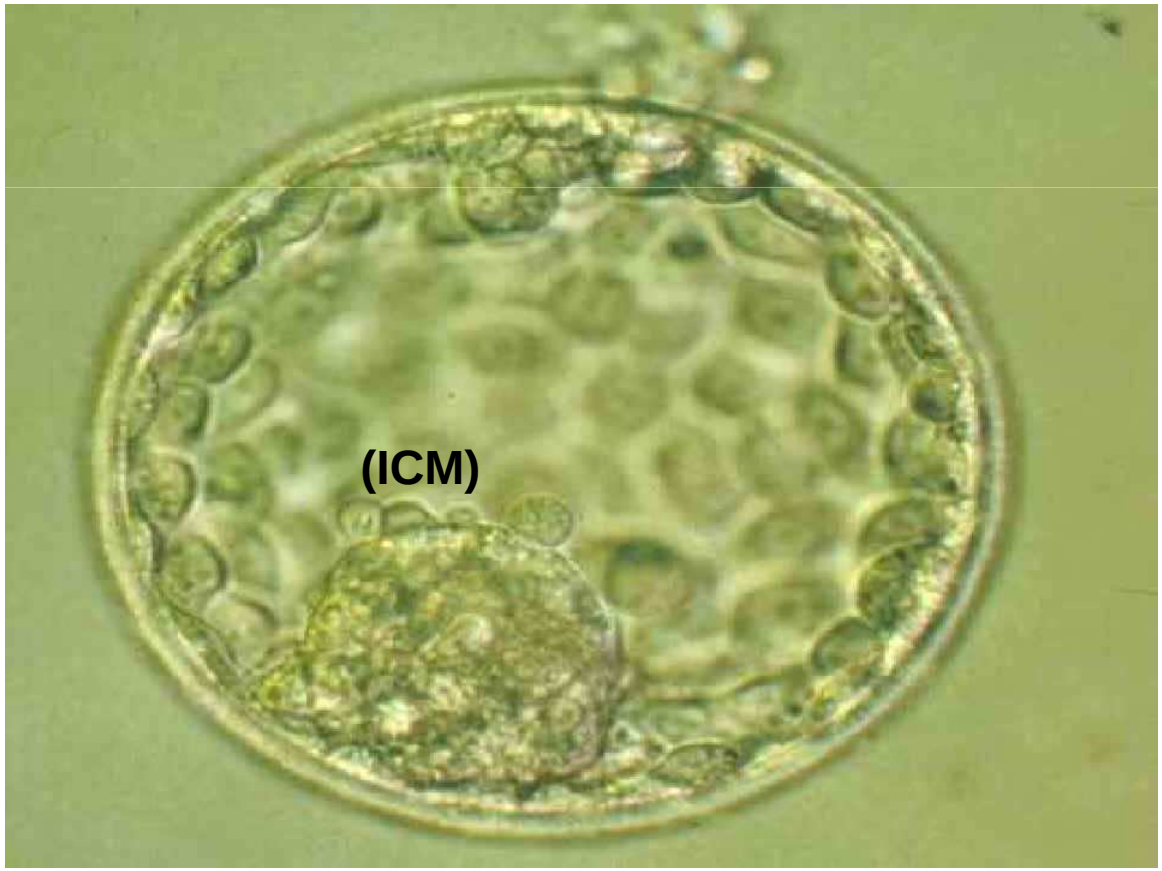
Día 5

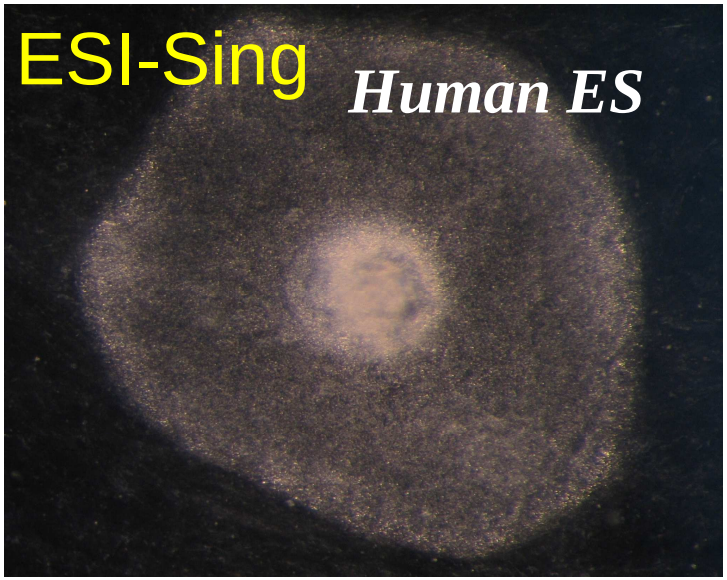


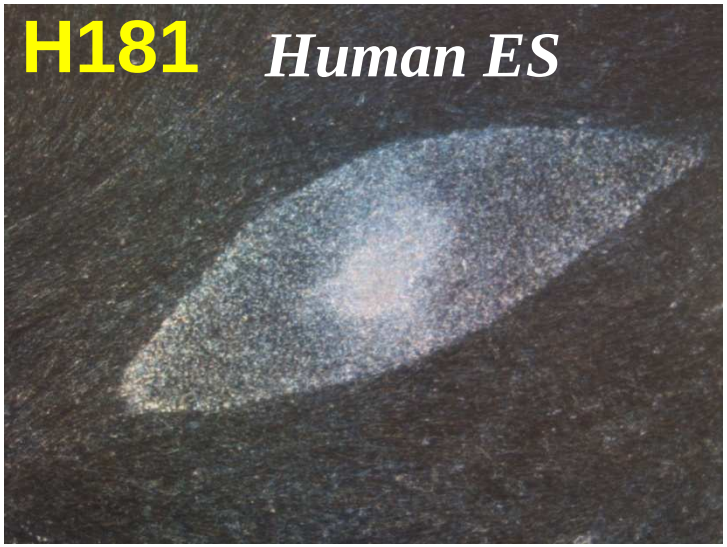
Día 6-7

Methylation Low

Masa Celular Interna:
baja silenciación de genes







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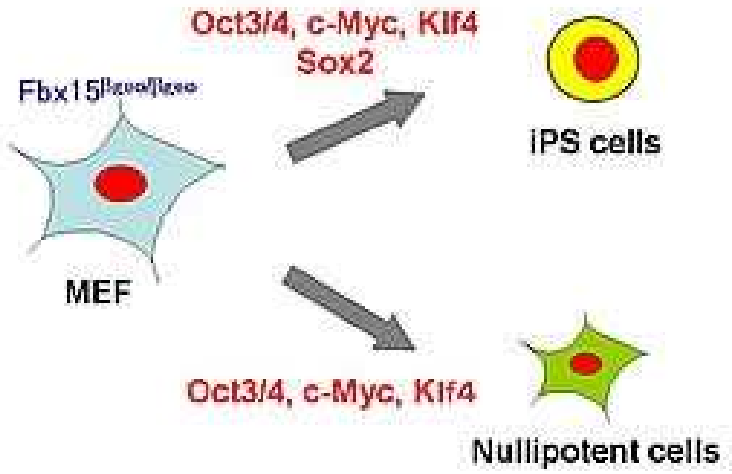
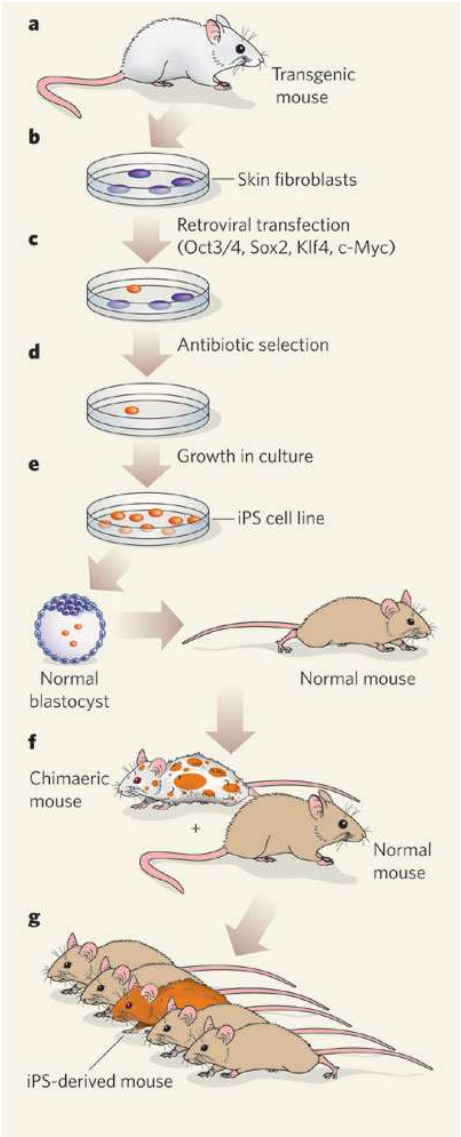
Células iPS

Yamanaka (2006)
Kyoto
Cell 126: 663-676





Adult Stem Cells

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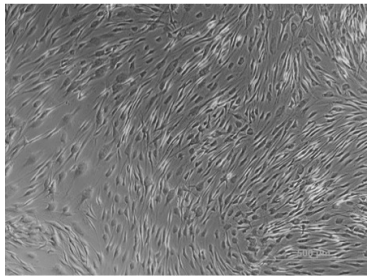
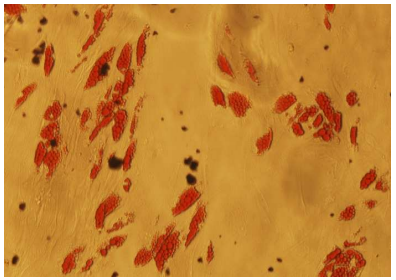
Mesenquimales

- *Médula Ósea*
- *Tejido adiposo*
- *Placenta*
- *Endometrio*
- *Pulpa dental, etc*

AdMSC



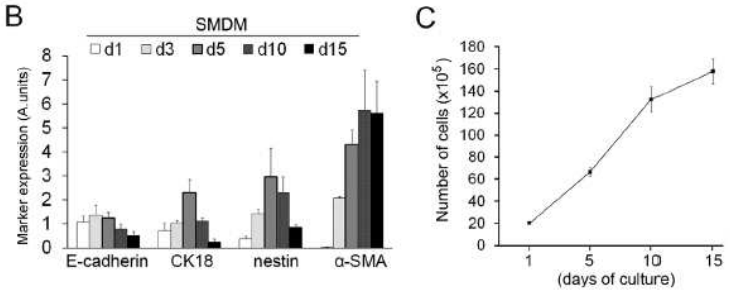
Osteogenesis

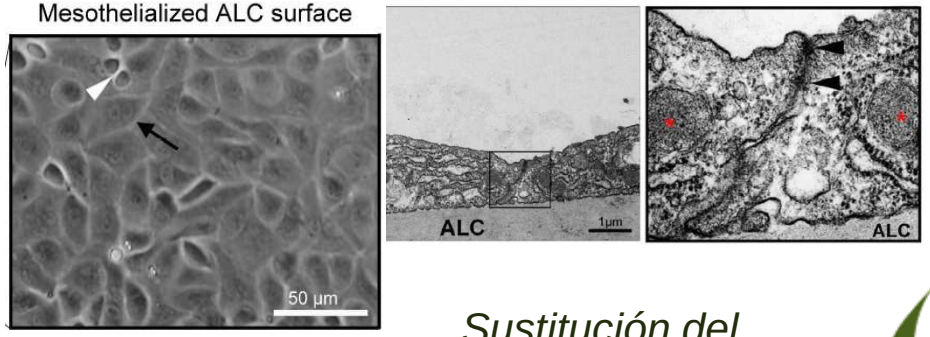


Mesoteliales

Functional Vascular Smooth Muscle-like Cells Derived from Adult Mouse Uterine Mesothelial Cells

Christian Claude Lachaud[1], Daniela Pezzolla[1], Alejandro Domínguez-Rodríguez[2], Tarik Smani[2], Bernat Soria[1,3*§], Abdelkrim Hmadcha[1,3*§]





Sustitución del Endotelio corneal

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Modificaciones Epigenéticas

OPEN ACCESS Freely available online

PLoS one

Cancer Genes Hypermethylated in Human Embryonic Stem Cells

Vincenzo Calvanese[1], Angelica Horrillo[2], Abdelkrim Hmadcha[2], Beatriz Suarez-Álvarez[3], Agustín F. Fernandez[1,4], Ester Lara[1], Sara Casado[1], Pablo Menendez[5], Clara Bueno[5], Javier Garcia-Castro[5], Ruth Rubio[5], Pablo Lapunzina[6], Miguel Alaminos[7], Lodovica Borghese[8], Stefanie Terstegge[8], Neil J. Harrison[9], Harry D. Moore[9], Oliver Brüstle[8], Carlos Lopez-Larrea[3], Peter W. Andrews[9], Bernat Soria[3], Manel Esteller[1,4]*, Mario F. Fraga[1,10]*

1 Cancer Epigenetics Group, Spanish National Cancer Research Centre (CNIO), Madrid, Spain, **2** Andalusian Center for Molecular Biology and Regenerative Medicine (CABIMER), Seville, Spain, **3** Unidad de Histocompatibilidad, HUCA, Oviedo, Spain, **4** Cancer Epigenetics and Biology Program (PEBC), Catalan Institute of Oncology (ICO), Barcelona, Spain, **5** Andalusian Stem Cell Bank (BACM)/University of Granada, Instituto de Investigaciones Biomédicas, Parque Tecnológico de la Salud, Granada, Spain, **6** S. de Genética Médica y Molecular, Hospital Universitario La Paz, Madrid y CIBERER, Centro de Investigación Biomédica en Red de Enfermedades Raras, Madrid, Spain, **7** Department of Histology, University of Granada, Granada, Spain, **8** Institute of Reconstructive Neurobiology, LIFE & BRAIN Center, University of Bonn and Hertie Foundation, Bonn, Germany, **9** Centre for Stem Cell Biology and the Department of Biomedical Science, University of Sheffield, Sheffield, United Kingdom, **10** Department of Immunology and Oncology, Centro Nacional de Biotecnología/CSIC, Darwin 3, Cantoblanco, Madrid, Spain

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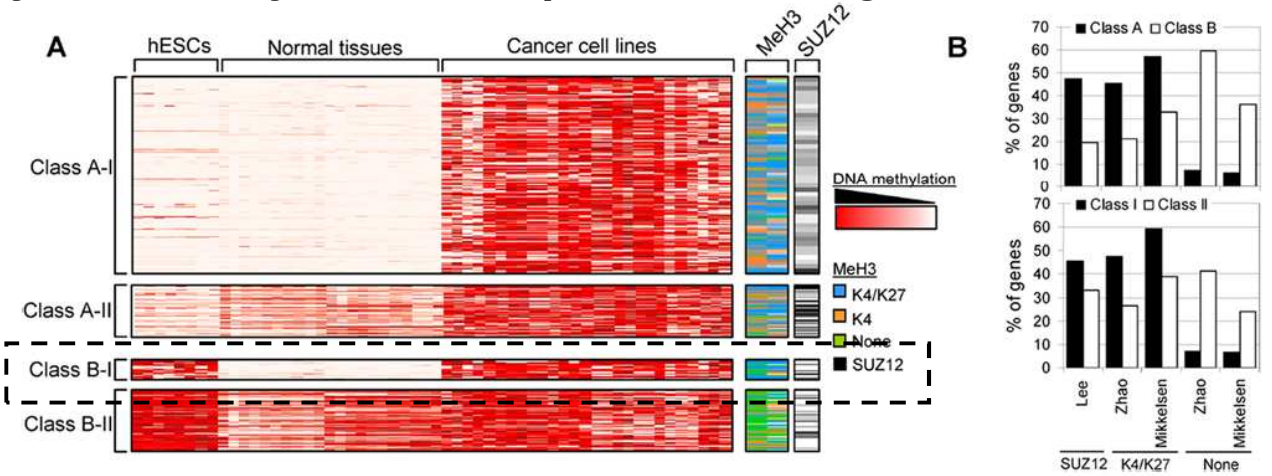
DNA Methylation

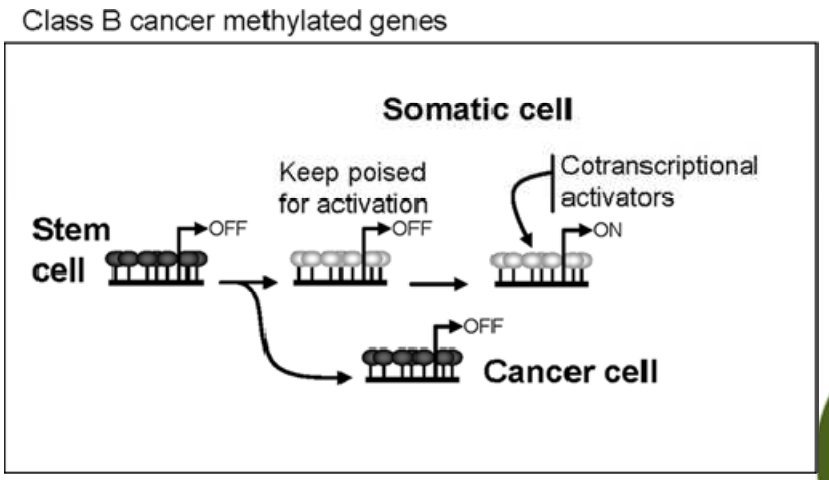
Goldengate methylation arrays: 1505 sequences / 807 genes



Cancer Hypermethylated Genes

	hESC	*Somatic*	*Cancer*	*Genes*
A-I	-	-	+	Early Differentiation
A-II	+/-	+/-	+	Tissue Specification
B-I	+	-	+	Early Differentiation (*)
B-II	+	+/-	+	Tissue Specification

(*) Proposed model for "aberrant methylation of cancer genes"



OPEN

Citation: Cell Death and Disease (2013) 4, e570; doi:10.1038/cddis.2013.88

www.nature.com/cddis

Zebularine regulates early stages of mESC differentiation: effect on cardiac commitment

A Horrillo[1], D Pezzolla[1,2], MF Fraga[3], Y Aguilera[1], C Salguero-Aranda[2,4], JR Tejedo[2,4], F Martin[2,4], FJ Bedoya[2,4], B Soria[1,2,5] and A Hmadcha*[,1,2,5]

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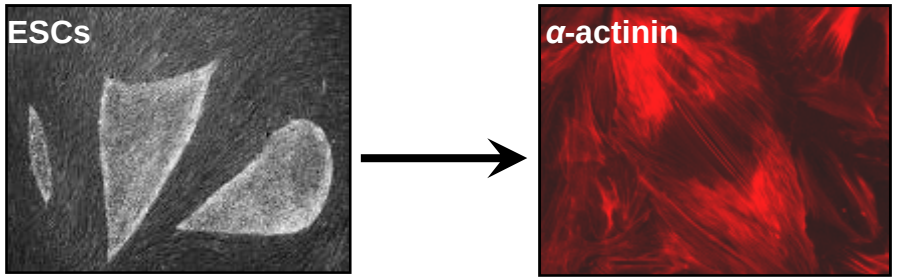

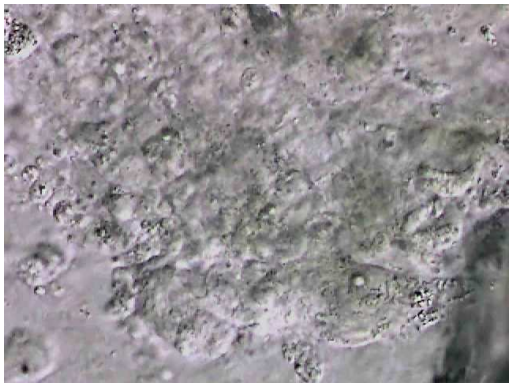

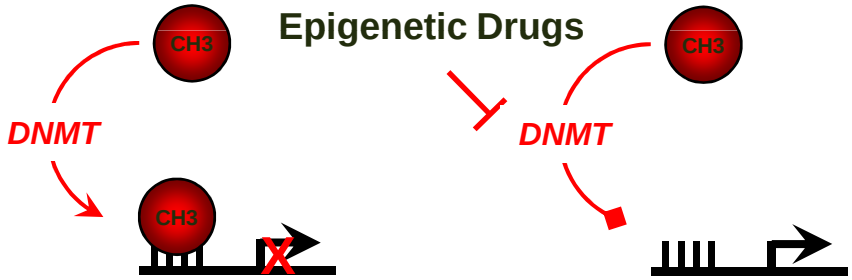

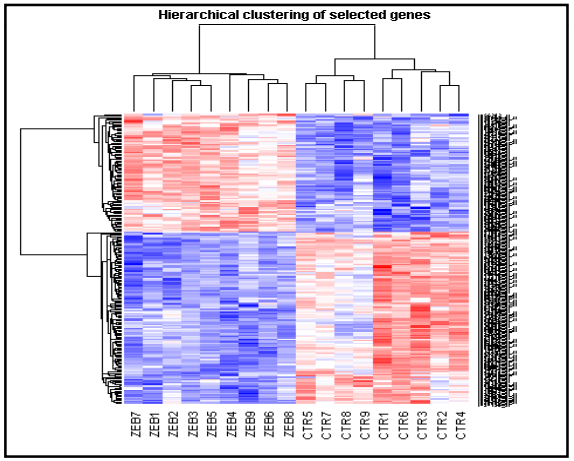

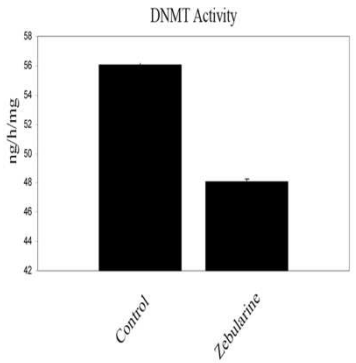

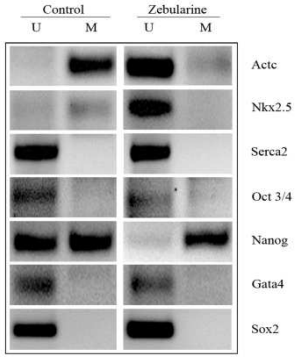

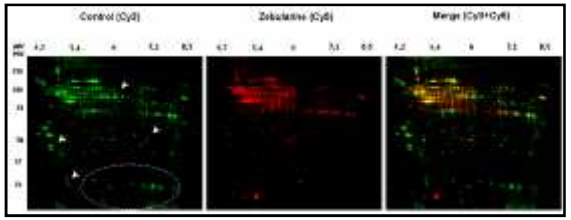

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- *Stem Cells and Regenerative Medicine*

- *Cellular Medicaments: a Change in the Paradigm*

- *Cell Therapy in Pediatric Diseases*

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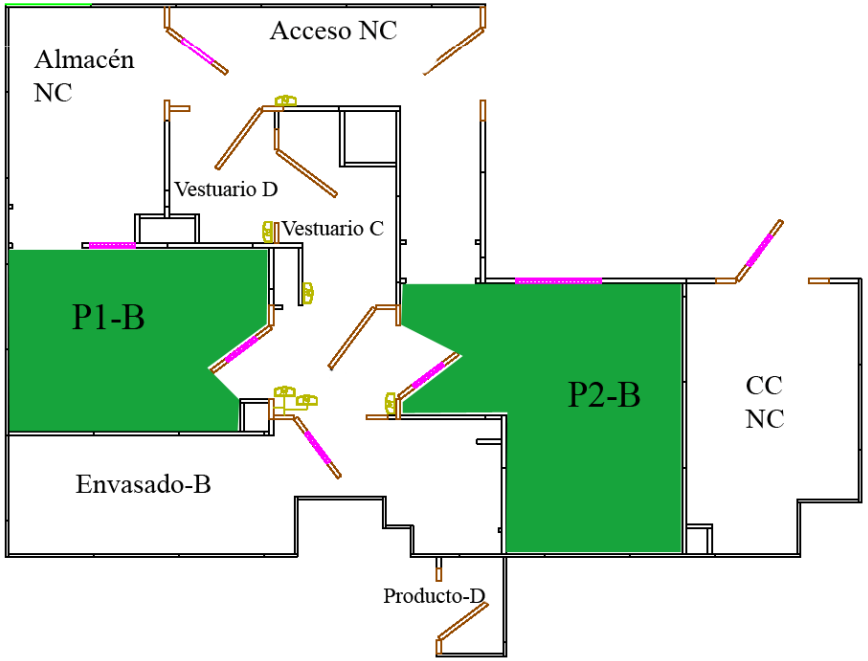
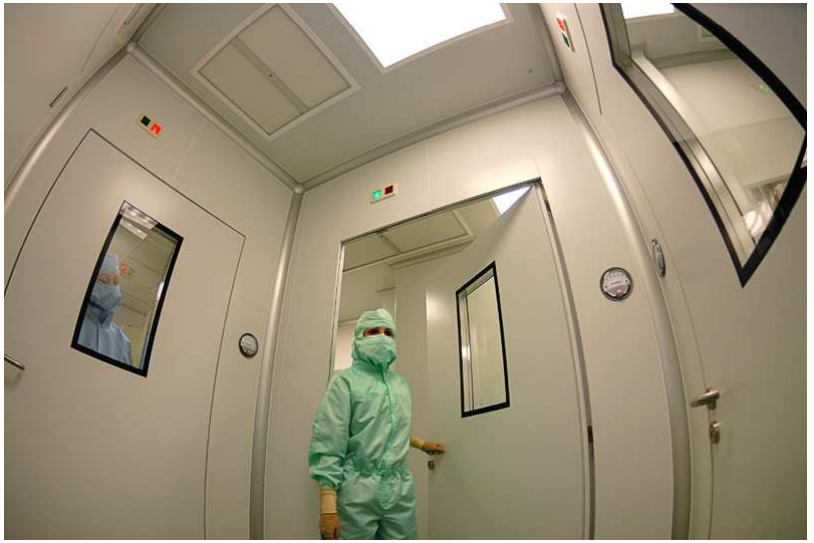
Cell Therapy of Diabetic Complications

GMP Cell Production Unit: GMP-CABIMER:

- First GMP Unit Certified by AEMPS in Andalucia (6th Nov 2009)
- Two Production Units
- Four Clinical Trials On-going (Diabetic foot and Múltiple Sclerosis)





Cells Tissues Organs

Cells Tissues Organs 2008;188:70–77
DOI: 10.1159/000119407

Cell Therapy for Diabetes Mellitus: An Opportunity for Stem Cells?

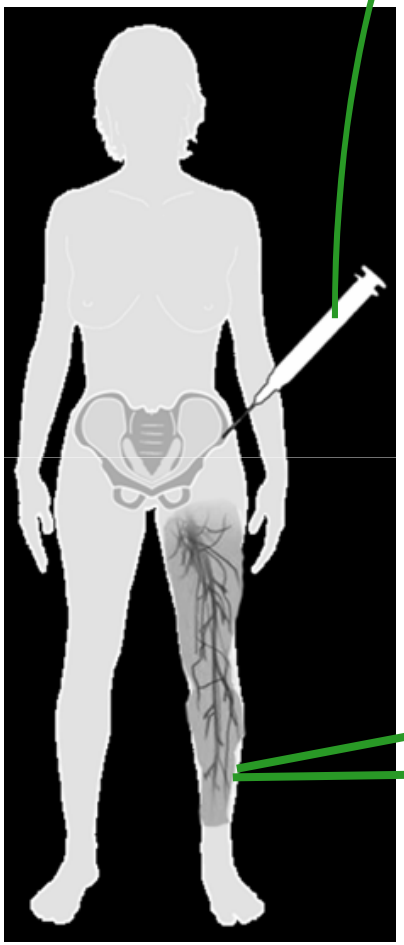
B. Soria[a, d] F.J. Bedoya[a] J.R. Tejedo[a] A. Hmadcha[a] R. Ruiz-Salmerón[b]
S. Lim[c, d] F. Martin[a]

Table 3. Current clinical trials using autologous stem cells for critical limb ischemia

Trial	Enrollment	Treatment	Injection	Population	Primary endpoint
UMC Utrecht, Utrecht, Holland	2006–2009	BMMNC	intraarterial	55–55	amputation
Indiana University, Bloomington, Ind., USA	2004–2007	BMMNC	intramuscular	20	MAE
Institute of Biomedical Research and Innovation, Kobe, Japan	2003–2008	PBMNC, CD34+	intramuscular	15	amputation
University of Naples, Naples, Italy	2005–2006	BMMNC		20	ABI, ulcer
Northwestern University, Chicago, Ill., USA	since 2004	hematopoietic stem cells		12	survival
Goethe University, Frankfurt, Germany	2005–2007	BMMNC	intraarterial	20–20	ABI

BMMNC = Bone marrow mononuclear cells; PBMNC = peripheral blood mononuclear cells; MAE = major adverse events at 12 weeks; ABI = ankle-brachial index.

Bone marrow



Mononuclear Fraction (Ficoll) +

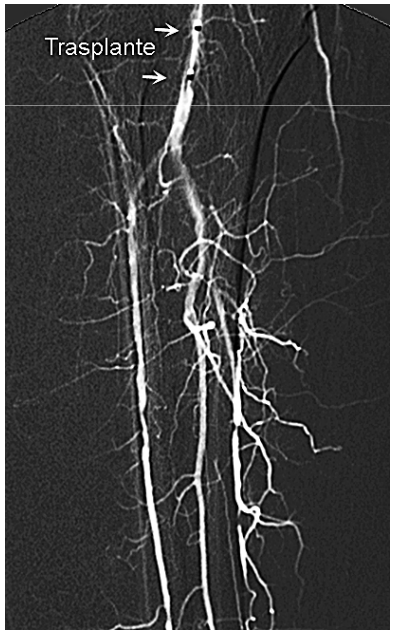
Cytometry $CD34^+$ (CABIMER)

Intraarterial Infusion
$113,7\text{-}434,5\ 10^6$ cells

Clinics

Angiography

MetaMorph
(CABIMER)

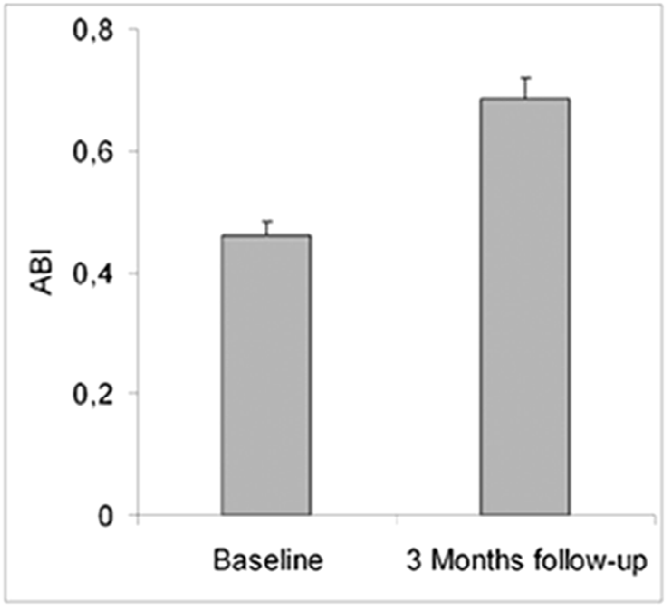


Cell Transplantation* 20(10) *1629-1639 (2011)

1.- CLINICS (reported by patients): 0-24 months

1.1 Temperature sensation increase
1.2 Pain decreases
1.3 Increase walking distance
1.4 Gastrocnemius perimeter increases

2.- ABI Increases



Cell Transplantation* 20(10) *1629-1639 (2011)

3.- ULCERS U.TEXAS Classification

(Lavery LA et al. J. Foot. Ankle. Surg. 35: 528-31. 1996)

			Baseline Cases (%)	3 Months Cases (%)	12 Months Cases (%)
University of Texas	Stage A	No Ulcer	1 (5%)	-	-
		A0	3 (15%)	15 (**79%**)	14 (**87,5%**)
		A1		2 (10,5%)	1(6,25%)
		A2	1 (5%)	1 (5,3%)	
		A3			
	Stage C	C0	3 (15%)		
		C1	3 (15%)		
		C2	3 (15%)		
		C3	6 (30%)		

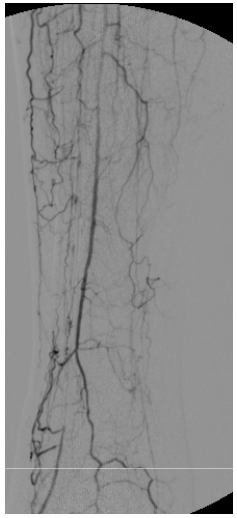
4.- CIL EVOLUTION

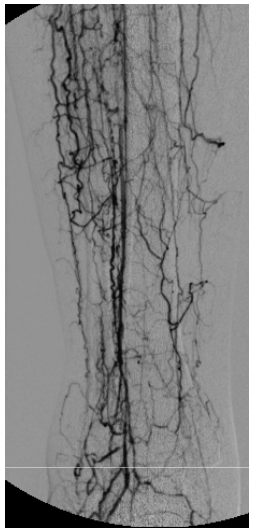
Rutherford-Becker Scale *(Rutherford RB et al. J. Vasc. Surg. 26:517-38. 1997)*

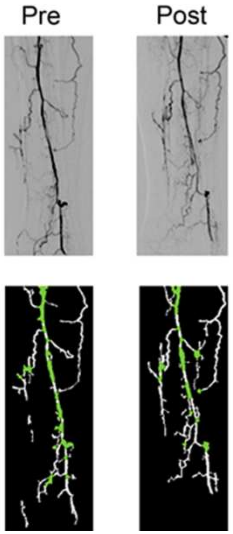
			Baseline Cases (%)	3 Months Cases (%)	12 Months Cases (%)
Rutherford-Becker		Cat 0		0	4 (25%)
		Cat 1		5 (26,4%)	9 (**56,25%**)
		Cat 2		12 (**63,1%**)	3 (18,75%)
		Cat 3			
		Cat 4	3 (15%)		
		Cat 5	11 (55%)	2 (10,5%)	
		Cat 6	6 (30%)		

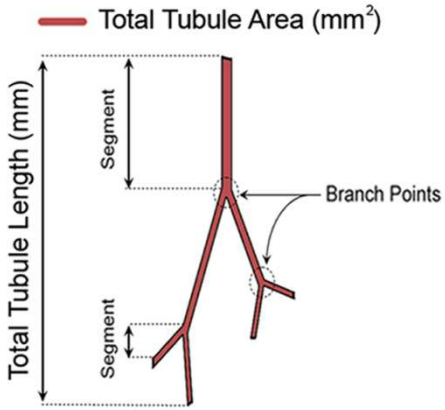
Cell Transplantation* 20(10) *1629-1639 (2011)

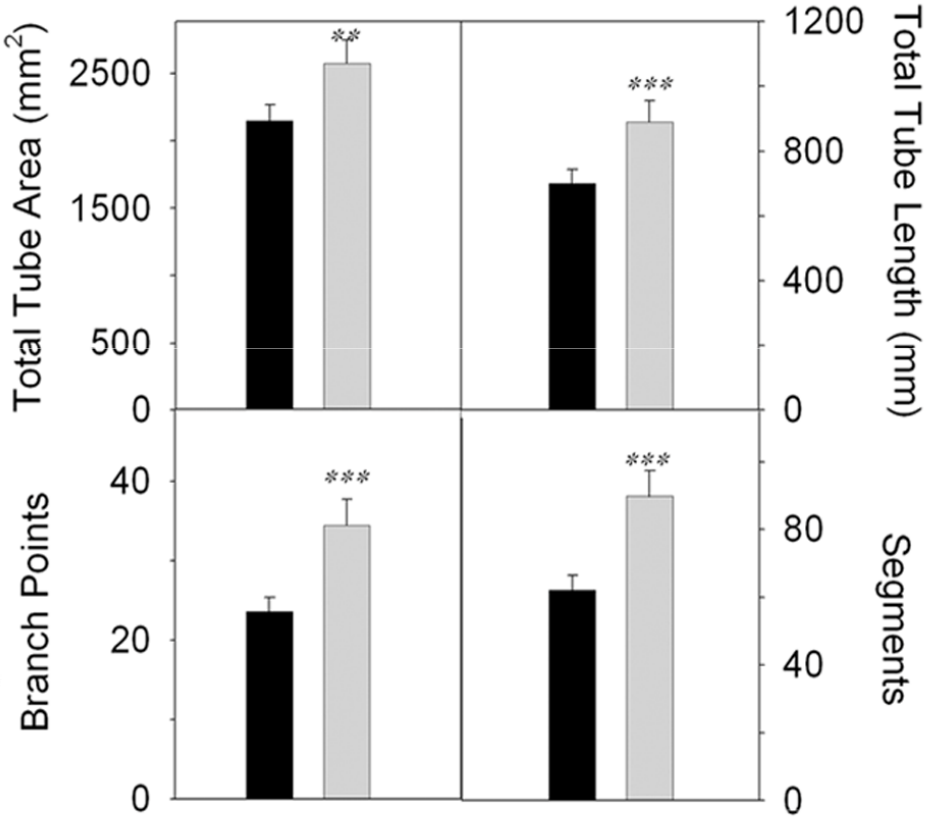
5.- ANGIOGRAPHY (Metamorph)











Cell Transplantation 20(10) 1629-1639 (2011)

UNDERSTANDING "CELLULAR MEDICAMENTS"

Small Molecules

(1600 …- XXth century)

↘

Biologicals (mAb, proteins)

(1922: insulin; mAb 80´s)

↘

Cells

(2000 -…)

UNDERSTANDING
"CELLULAR MEDICAMENTS"

USE OF MESENCHYMAL STEM CELLS IN THE TREATMENT OF THE CRITICAL ISCHAEMIA OF THE LIMB
BACKGROUND

1. **MSC promote fibrinolysis** *(Craig K et al, 2007 Antithrombogenic properties of BM-MSC … PNAS 29: 11915-20*)

.

2. **T2 Diabetes Mellitus patients present prothrombotic state (*Knudsen EC et al. 2011 J Thromb Haemost and Ay L et al. 2011 Eur J Clin Invest*)**

Acosta et al (2013) Adipose mesenchymal stromal cells isolated from type 2 diabetic patients display reduced fibrinolytic activity. Diabetes. 2013 Sep 16. [Epub ahead of print]

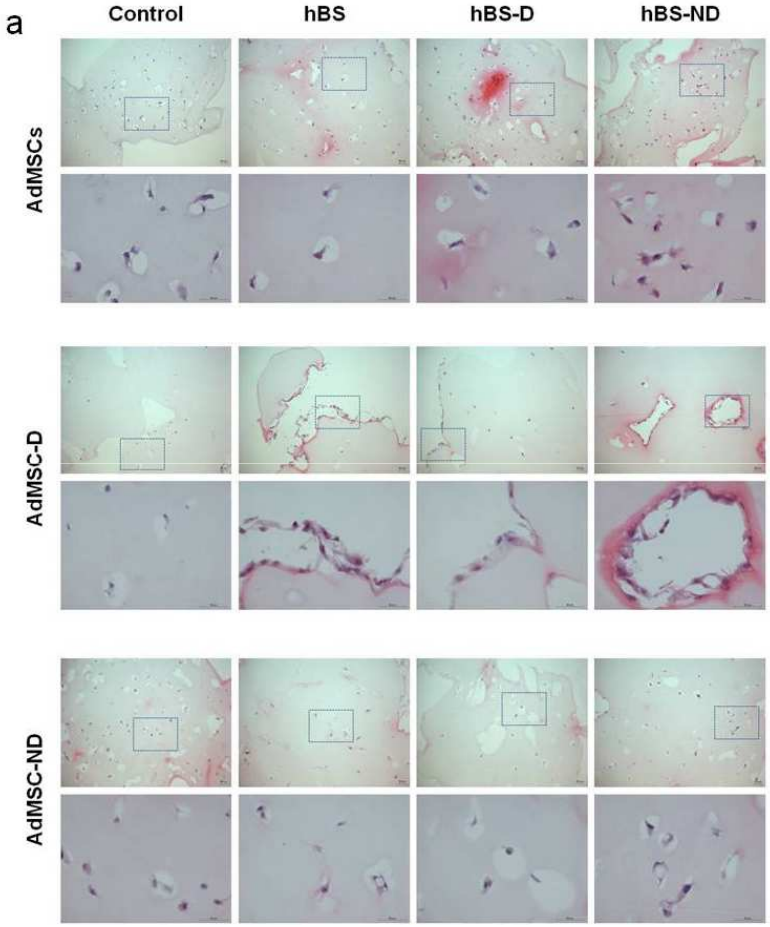
UNDERSTANDING "CELLULAR MEDICAMENTS"

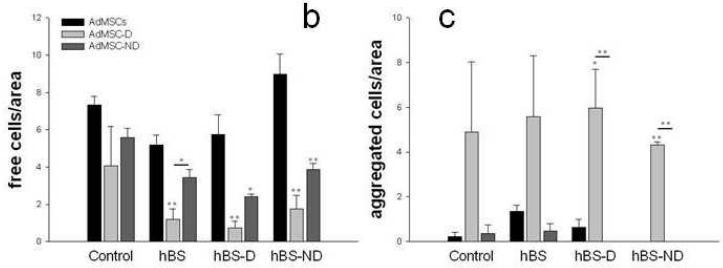
USE OF MESENCHYMAL STEM CELLS IN THE TREATMENT OF THE CRITICAL ISCHAEMIA OF THE LIMB

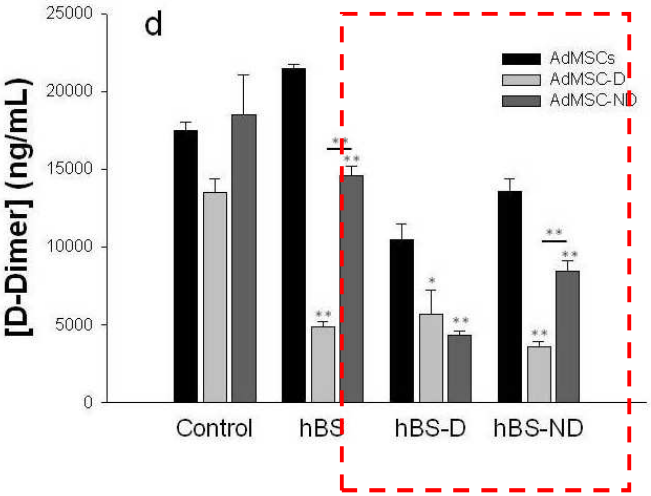
But, in 2010 we observed one adverse effects in two patients: MICROTHROMBOSIS that could be reversed by aggressive thrombolytic therapy

Acosta et al (2013) Adipose mesenchymal stromal cells isolated from type 2 diabetic patients display reduced fibrinolytic activity. Diabetes. 2013 Sep 16. [Epub ahead of print]

UNDERSTANDING "CELLULAR MEDICAMENTS"

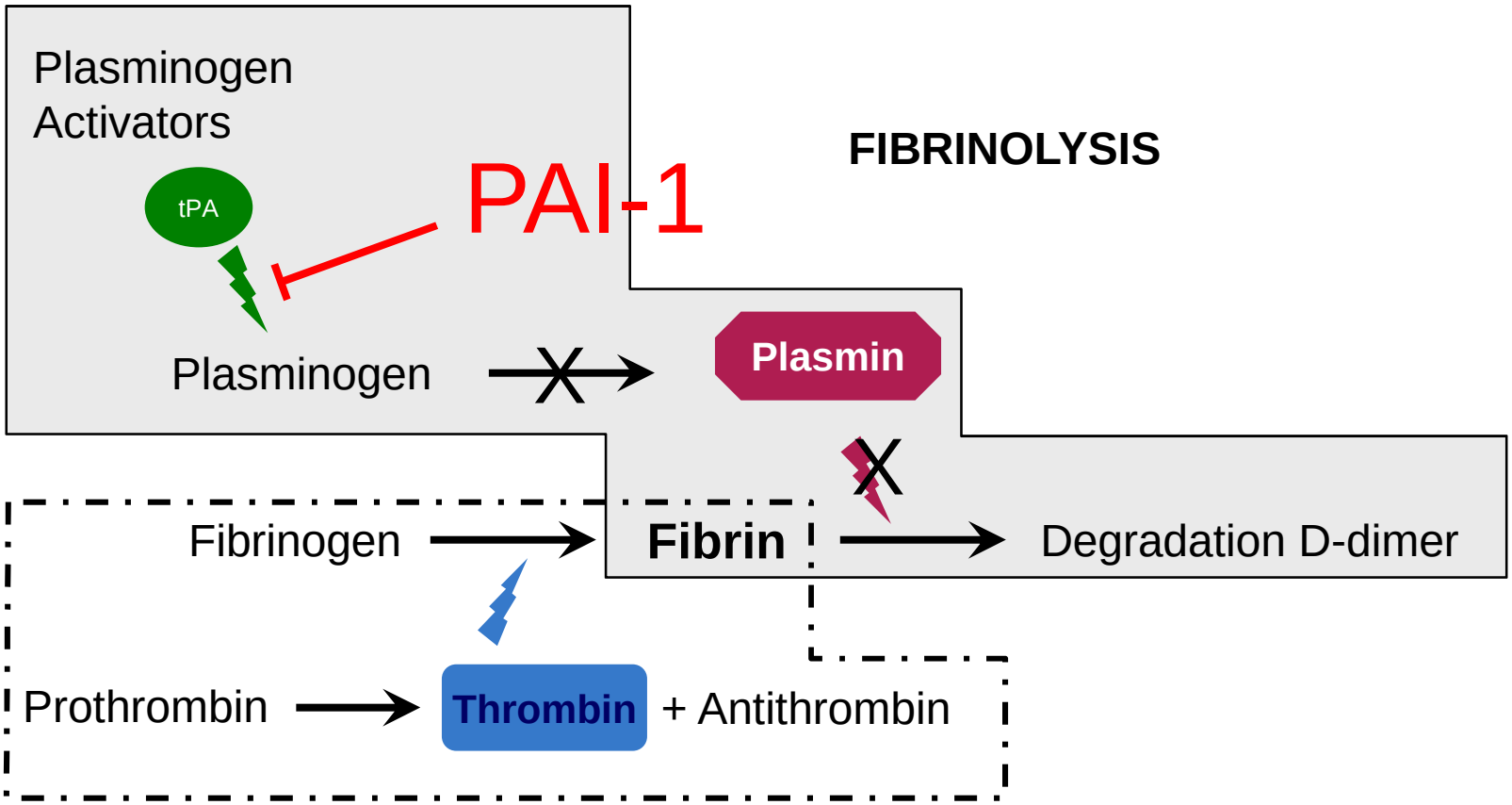






Acosta et al (2013) Adipose mesenchymal stromal cells isolated from type 2 diabetic patients display reduced fibrinolytic activity. Diabetes. 2013 Sep 16. [Epub ahead of print]

Fibrinolysis and Coagulation



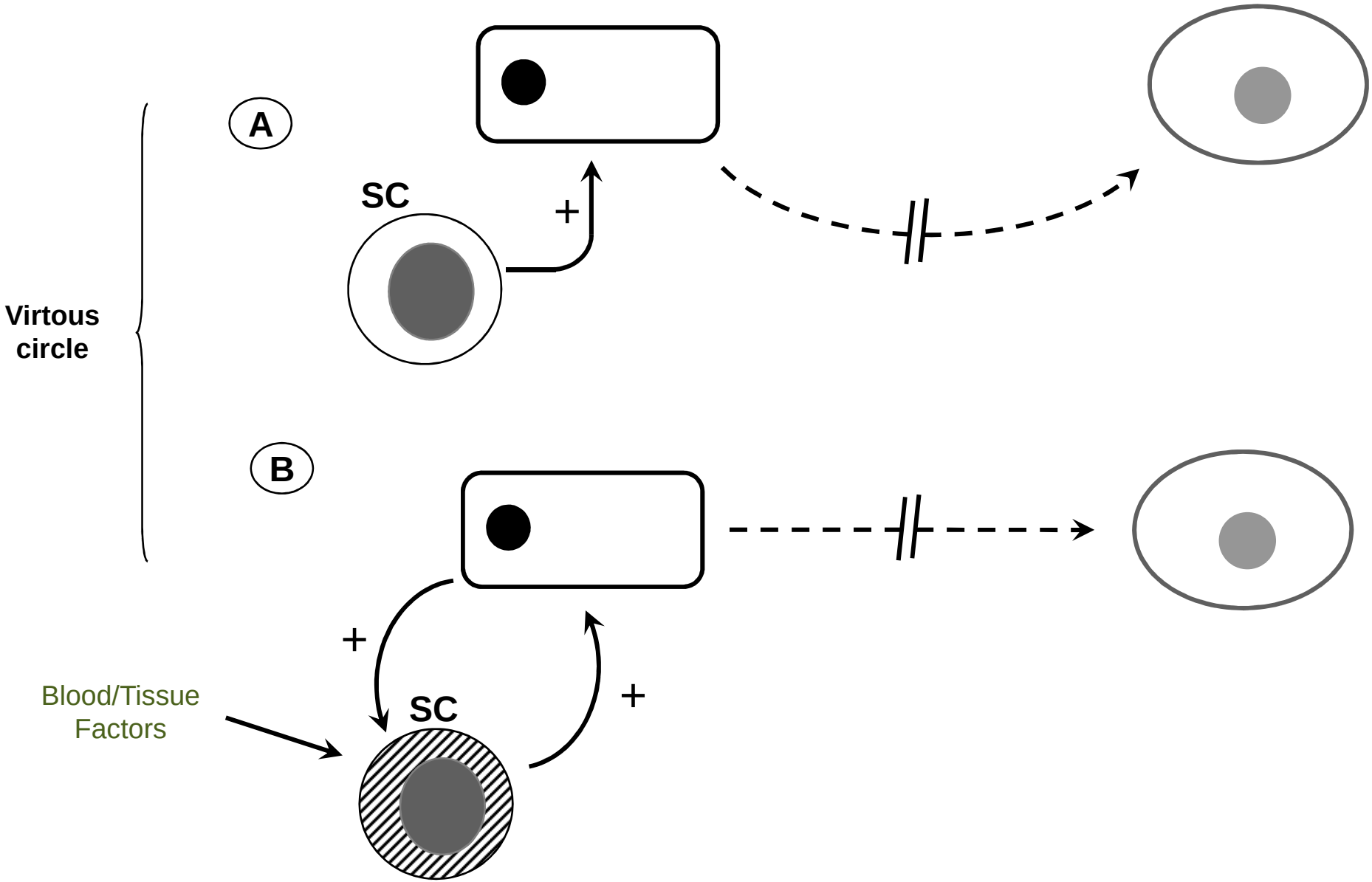
COAGULATION +++

tPA: tissue Plasminogen Activator
PAI-1: Plasminogen Activator Inhibitor

↑*PAI-1*, ↓*tPA*, ↓ *D-dimer*

	Control Serum	CIL-ND Serum	CIL-D Serum
AdMSC-C	+	+	++
Ad-MSC-ND	+	+	++
AdMSC-D	++	++	+++

SC Break the vicious circle and reset the situation



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- *Stem Cells and Regenerative Medicine*
- *Cellular Medicaments: a Change in the Paradigm*

- *Cell Therapy in Pediatric Diseases*

Cell Therapy in Pediatric Diseases

NOT INCLUDED- Hematopoietic stem cell transplantation: bone marrow, cord blood

1. *Type 1 Diabetes Mellitus*
2. *Child Autoimmune Diseases*
3. *Liver Diseases*
4. *Osteogenesis Imperfecta*
5. *Muscular Dystrophies*
6. *Neurological and Neurodegenerative Disorders*
7. *Future*

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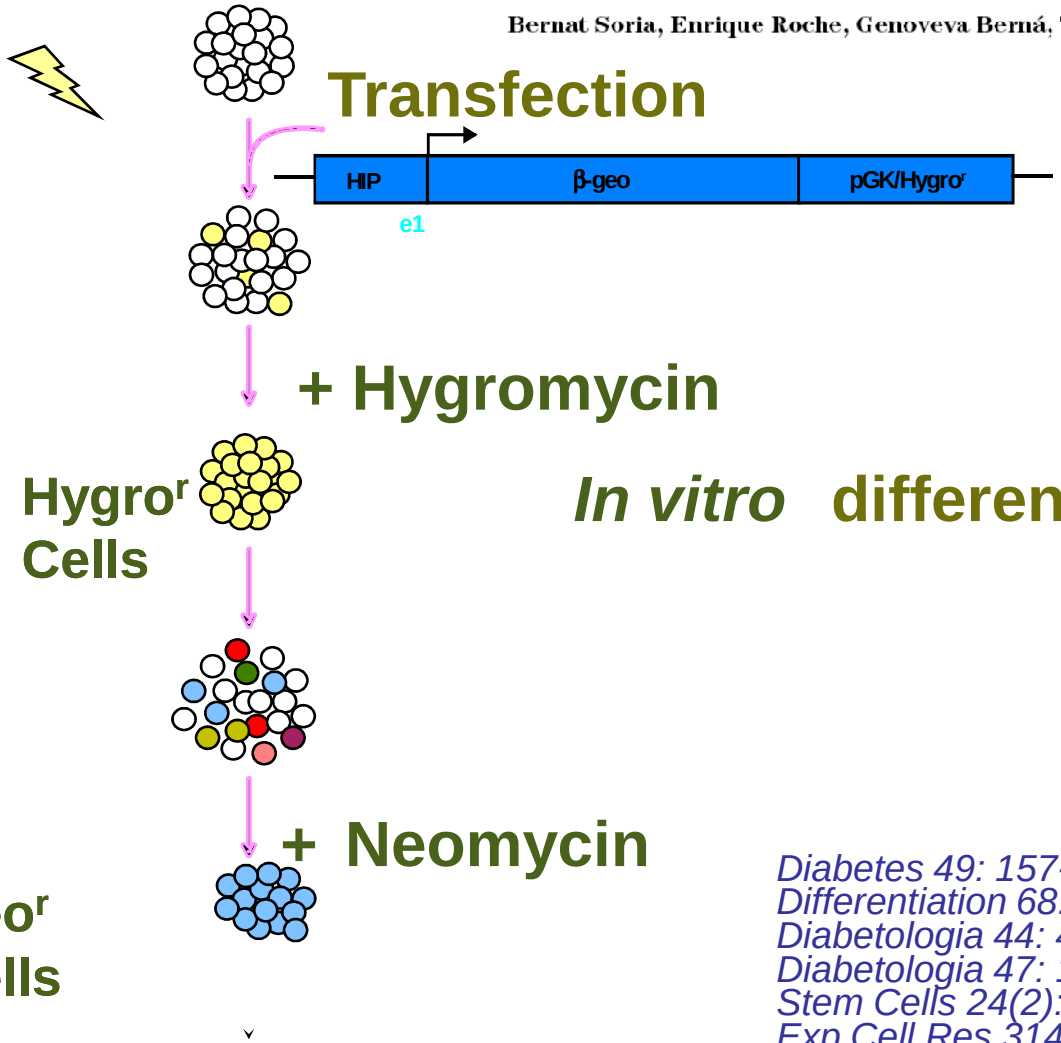
Type 1 DM

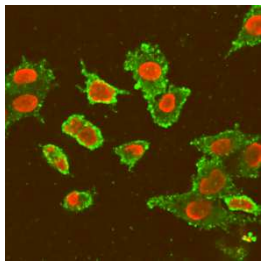
Qué tienen estas personas en común …

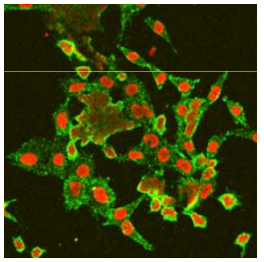
DIABETES

Insulin-Secreting Cells Derived From Embryonic Stem Cells Normalize Glycemia in Streptozotocin-Induced Diabetic Mice

Bernat Soria, Enrique Roche, Genoveva Berná, Trinidad León-Quinto, Juan A. Reig, and Franz Martín



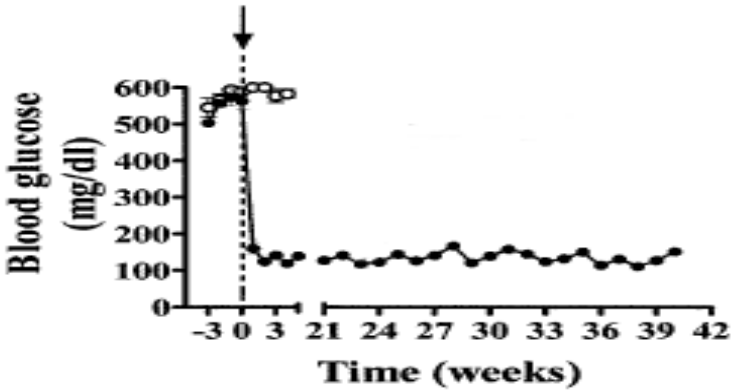




Diabetes 49: 157-162 (2000)
Differentiation 68: 4-5 (2001)
Diabetologia 44: 407-415 (2001)
Diabetologia 47: 1442-1451 (2004)
Stem Cells 24(2): 258-265 (2006)
Exp Cell Res 314: 969-974 (2008)

Nº citas: 1.052 (Oct 2013)

Insulin-producing cells normalize glycaemia in STZ-diabetic mice



Diabetes 49: 157-162 (2000)
Diabetologia 47: 1442-1451 (2004)
Stem Cells 24(2): 258-265 (2006)
Exp Cell Res 314: 969-974 (2008)

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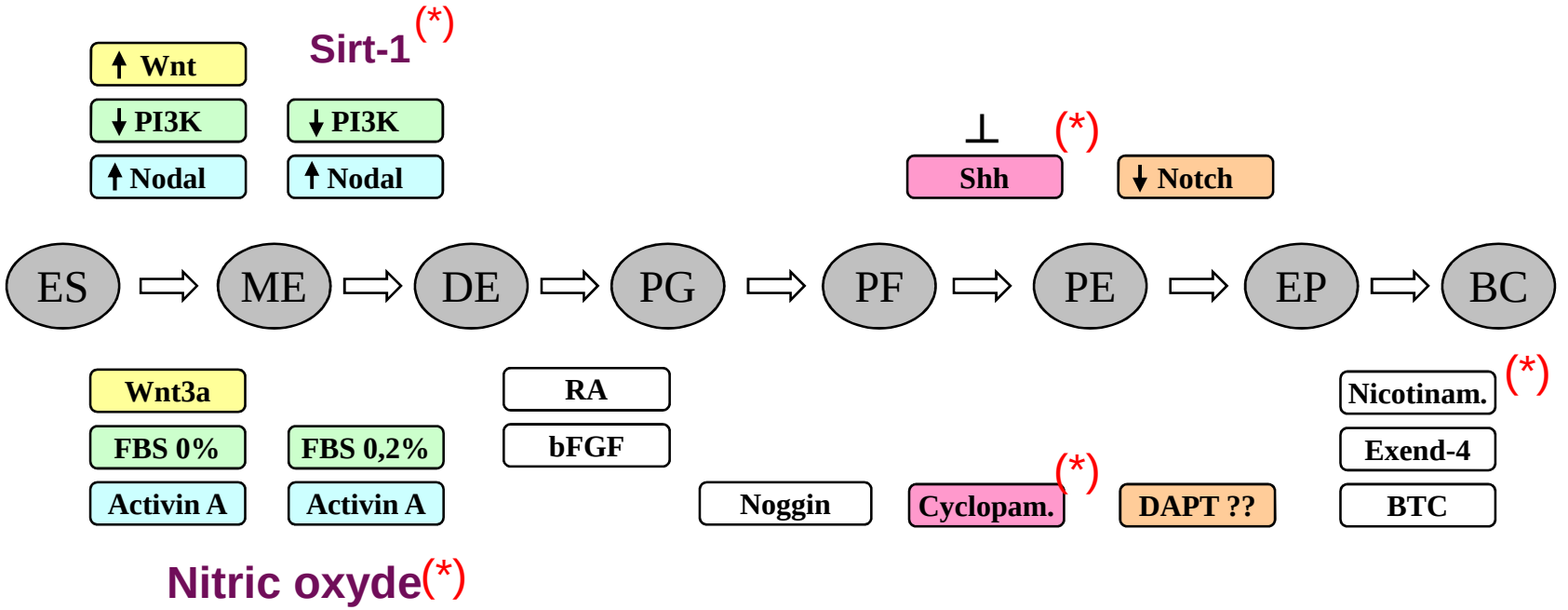
& MEDICINA REGENERATIVA

Insulin secreting cells from human ESC and iPSC

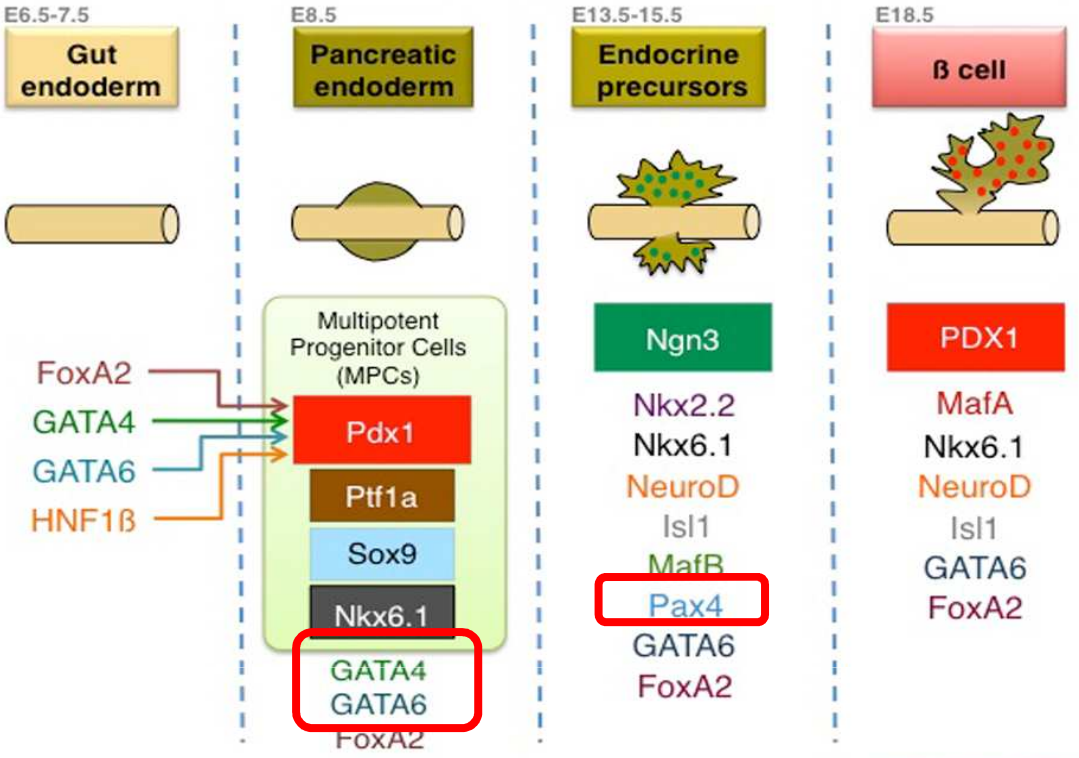
Design of new differentiation protocols (hESC/iPSC)

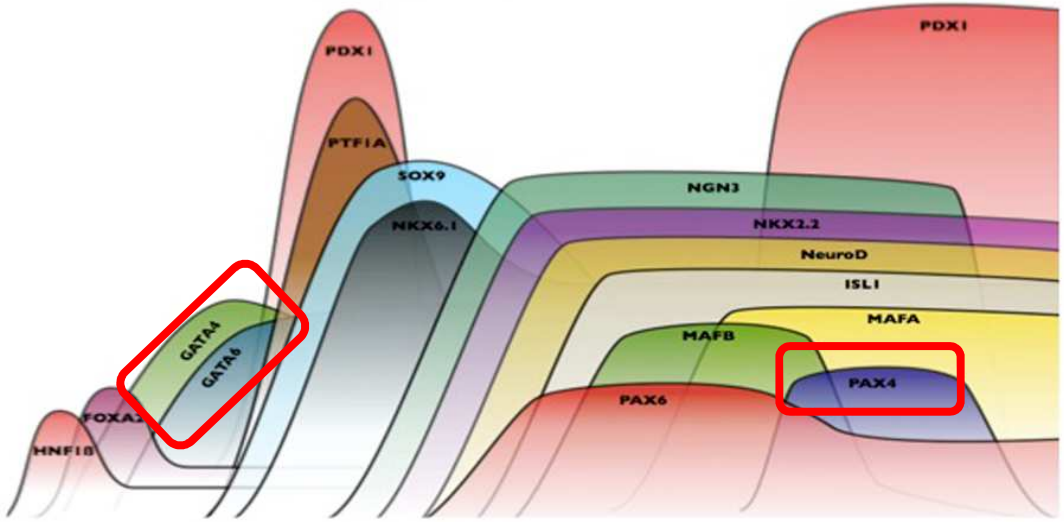


ES: embryonic stem cells; **ME**: mesendoderm; **DE**: definitive endoderm; **PG**: primitive gut; **PF**: posterior foregut; **PE**: pancreatic endoderm; **EP**: endocrine precursors; **BC**: beta cells;

DAPT: N-[N-(3,5-difluorophenacetyl)-L-alanyl-S-phenylglycine t-butyl ester; **BTC**: betacelulin.

1. Developmental Approach





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Research article Related Commentary, page 3469

GATA4 and GATA6 control mouse pancreas organogenesis

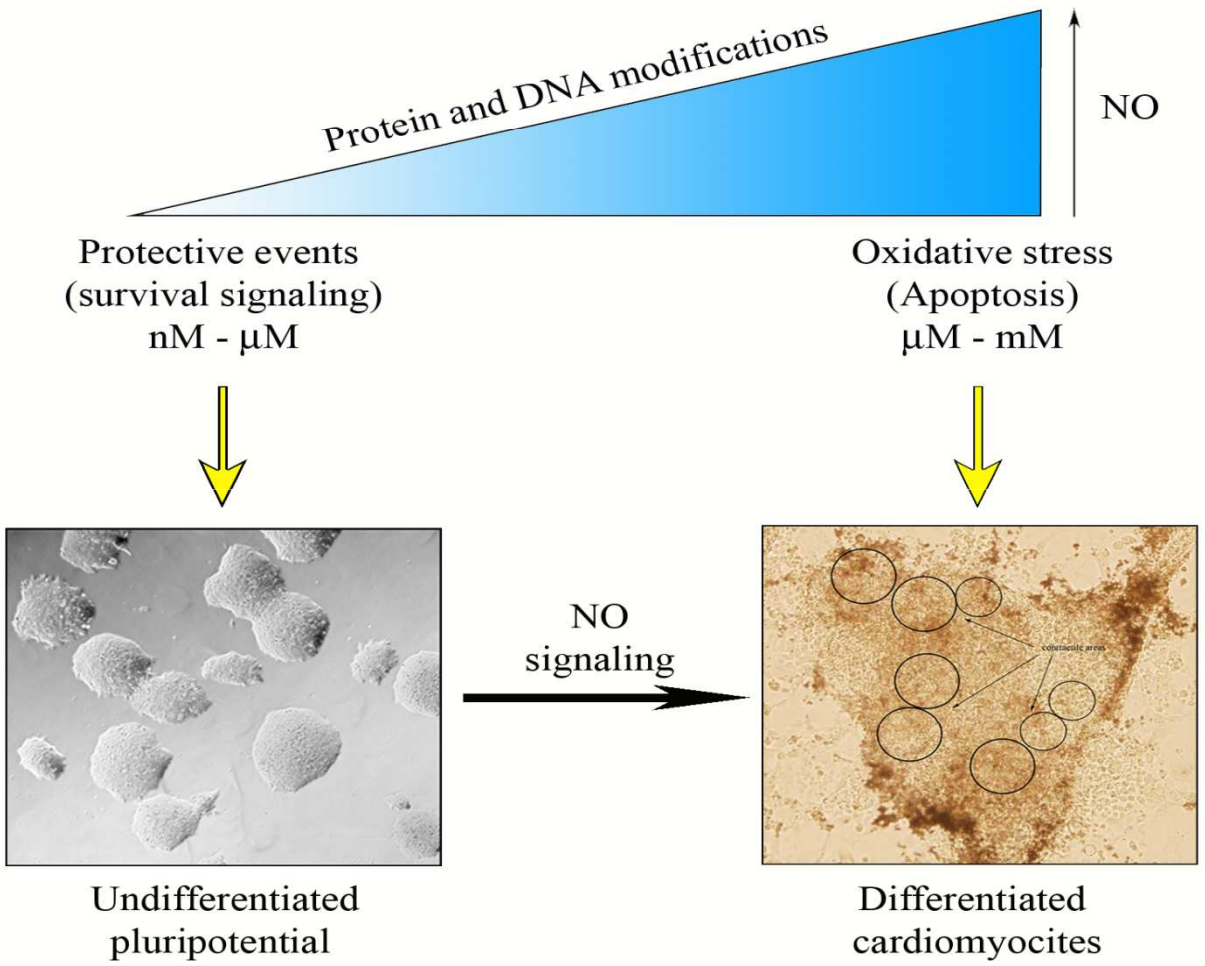
Manuel Carrasco, Irene Delgado, Bernat Soria, Francisco Martín, and Anabel Rojas

Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), Sevilla, Spain.
Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Barcelona, Spain.

2. “Small Molecule” Approach

a. *Nitric oxyde*
b. *Sirt-1*
c. *Resveratrol*

DUAL ROLE OF NITRIC OXIDE



Modified from Hess D.T. et al. Nat Rev Mol Cell Biol. 2005 Feb;6(2):150-66.

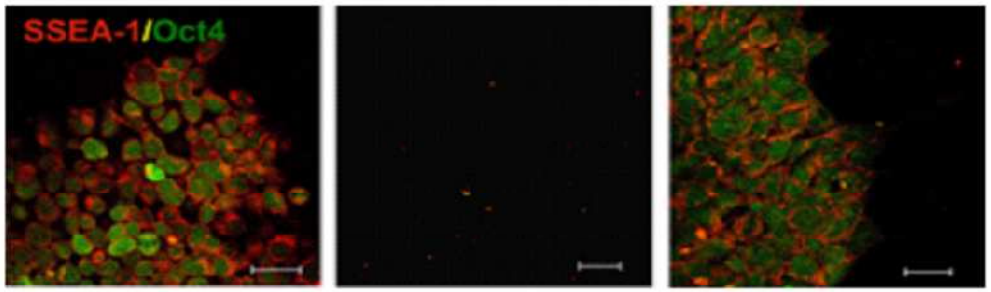
Citation: Cell Death and Disease (2010) 1, e80; doi:10.1038/cddis.2010.57

www.nature.com/cddis

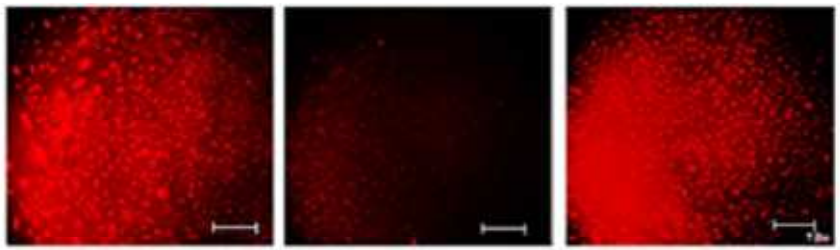
Low concentrations of nitric oxide delay the differentiation of embryonic stem cells and promote their survival

JR Tejedo*[,1], R Tapia-Limonchi[2], S Mora-Castilla[1], GM Cahuana[1], A Hmadcha[2], F Martin[1], FJ Bedoya[1] and B Soria[2]

Mouse ESC



Human ESC



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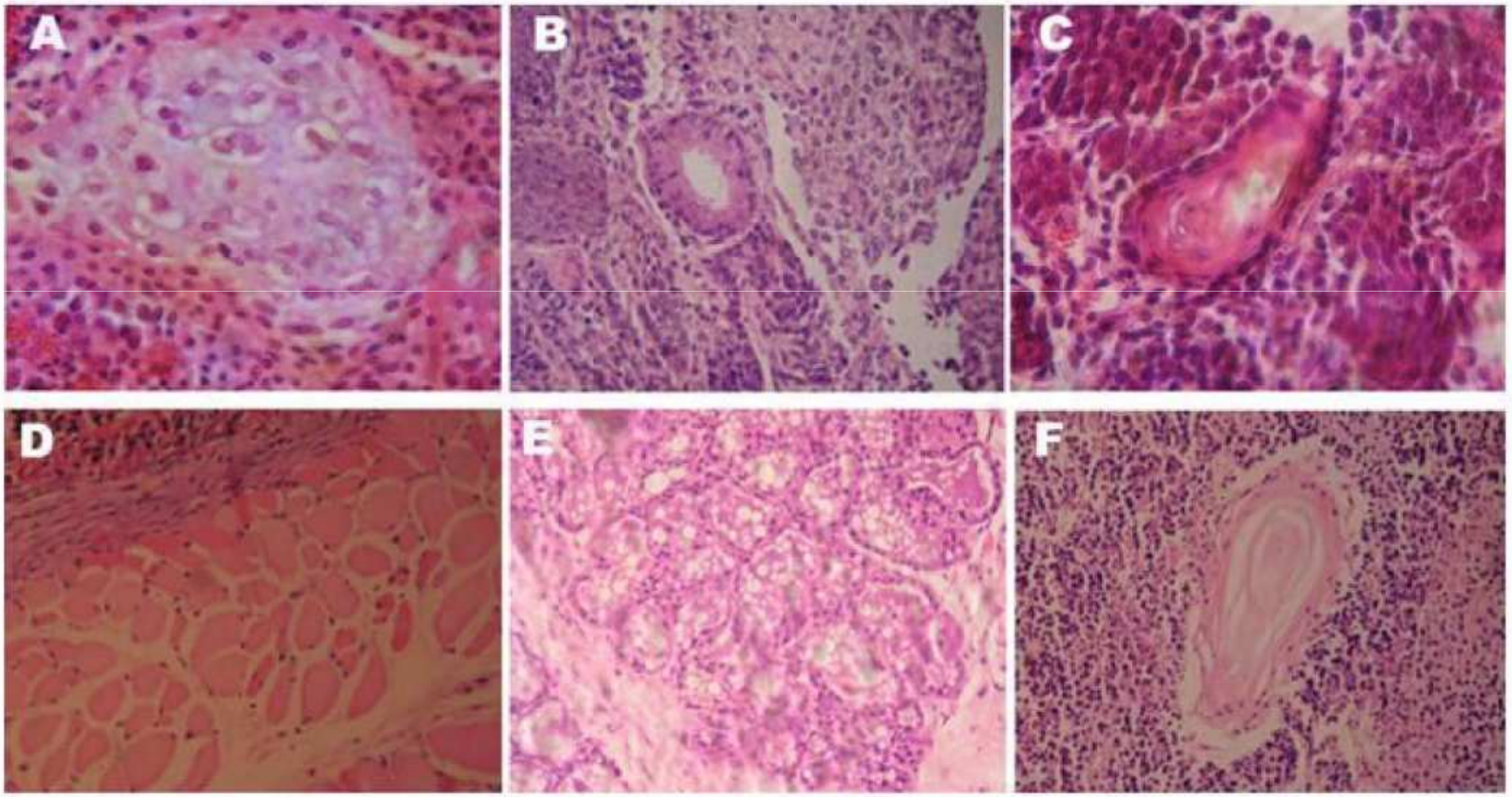
Teratome formation by mouse and human ESC cultured in low NO



Tejedo et al (2010) Cell Death Disease

CELL DEATH AND DIFFERENTIATION: 17(6): 1025-1033: 2010

Cell Death and Differentiation (2010) 17, 1025–1033

www.nature.com/cdd

Nitric oxide repression of Nanog promotes mouse embryonic stem cell differentiation

S Mora-Castilla[1], JR Tejedo[1], A Hmadcha[2], GM Cahuana[1], F Martín[1], B Soria[2] and FJ Bedoya*[,1]

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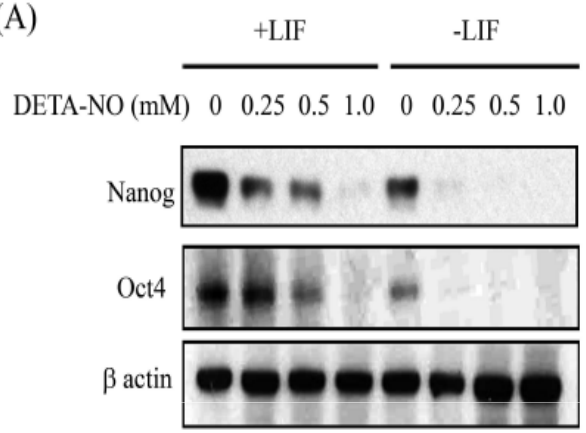
Nitric Oxide
High concentrations

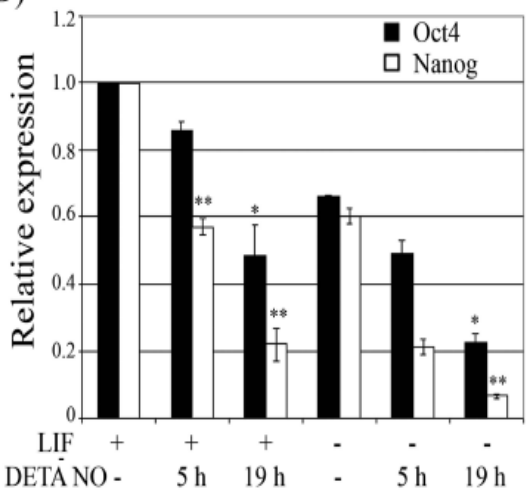
¿Mechanism?

Nanog
Oct 4

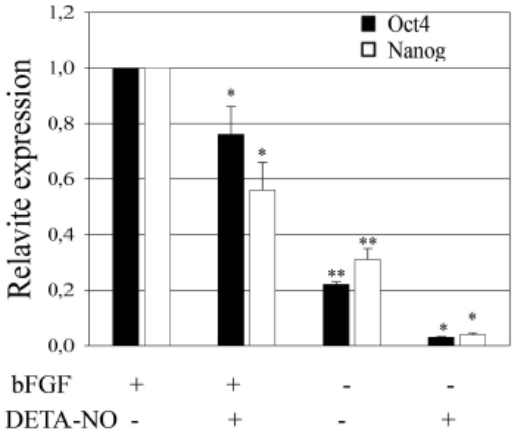
(A)



(D)



(F)



Mechanism of NO inhibition of Nanog:

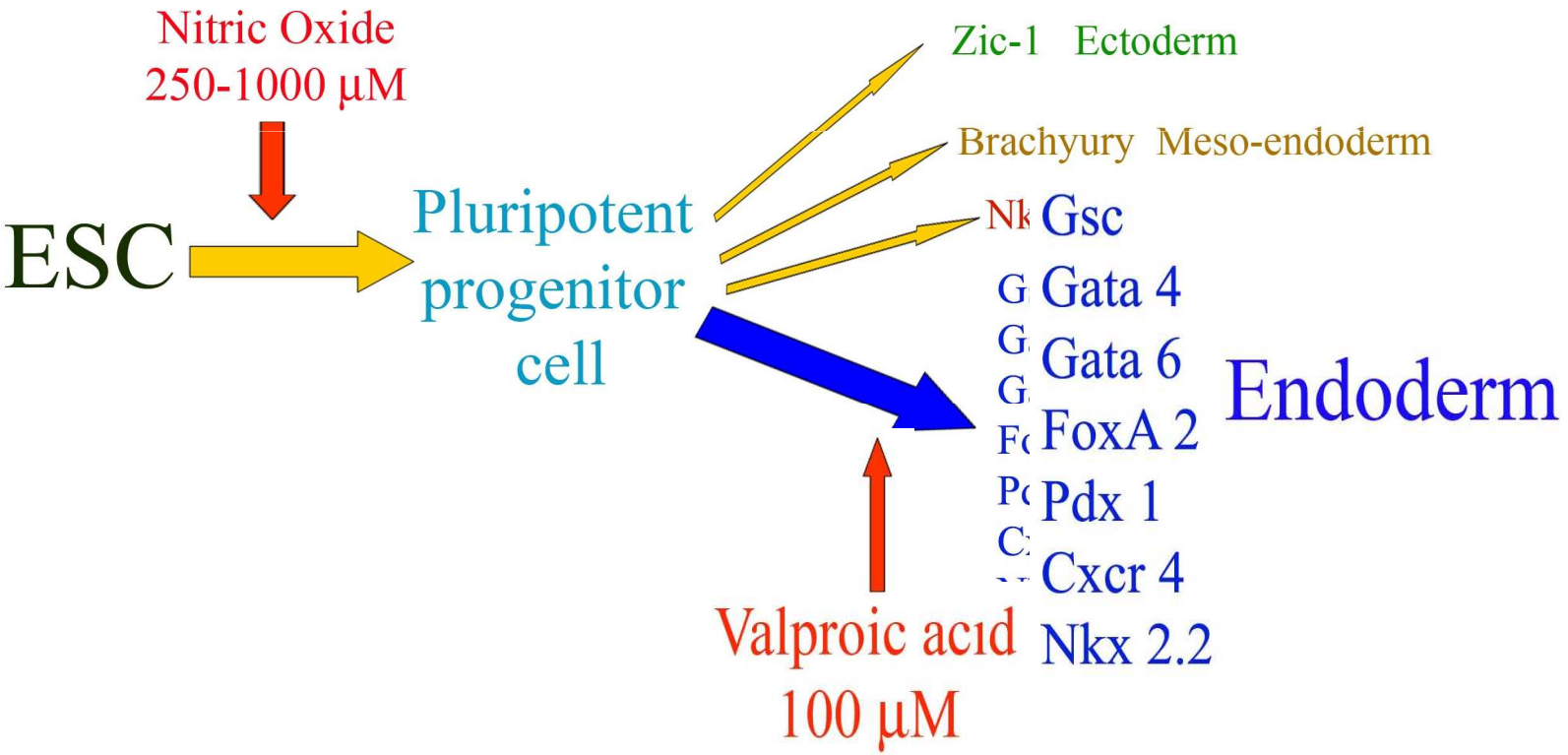
- Inhibits MDM2-dependent p53 degradation

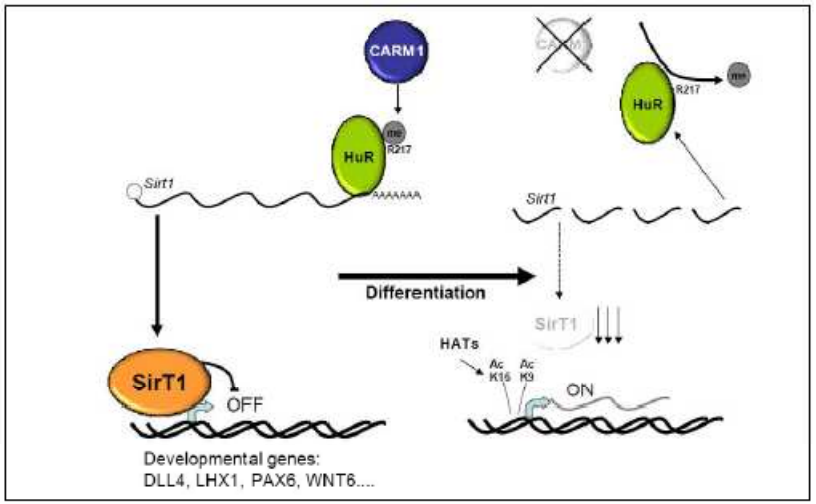
- Activation of p53 repressor protein by covalent modifications

- Binding of p53^{Ser315} to Nanog promoter region

NO *induces:*

Resistance to apoptosis and endoderm phenotype





Model for SirT action on developmental genes promoter during hESC differentiation. In pluripotent hESCs, CARM1 methylation of HUR increases HuR/SirT1 binding and, consequently, the SirT1 mRNA stability and SirT1 protein level. Under these conditions, SirT1 binds to the promoter and epigenetically represses specific developmental genes such us DLL4, LHX1, PAX6 and WNT6. In EBs, the decrease of CARM1 is associated with a decrease of HuR methylation and, consequently, of HuR/SirT1 binding, which results in less SirT1 mRNA and protein and the epigenetic reactivation of its target developmental genes.

Sirtuin 1 regulation of developmental genes during differentiation of stem cells

Calvanese et al (2010) PNAS USA 107:13736-13741

13736–13741 | PNAS | August 3, 2010 | vol. 107 | no. 31

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Day 0 1 2 5 8 11 20 22

Wnt3a [25ng/ml]

Activin A [100ng/ml]

RA [2 µM] bFGF [64 ng/ml]

Noggin [50 ng/ml]

Cyclopamine [0,25 µM]

Resveratrol [50 µM]

0% 0,2% 2%

RPMI

Definitive endoderm

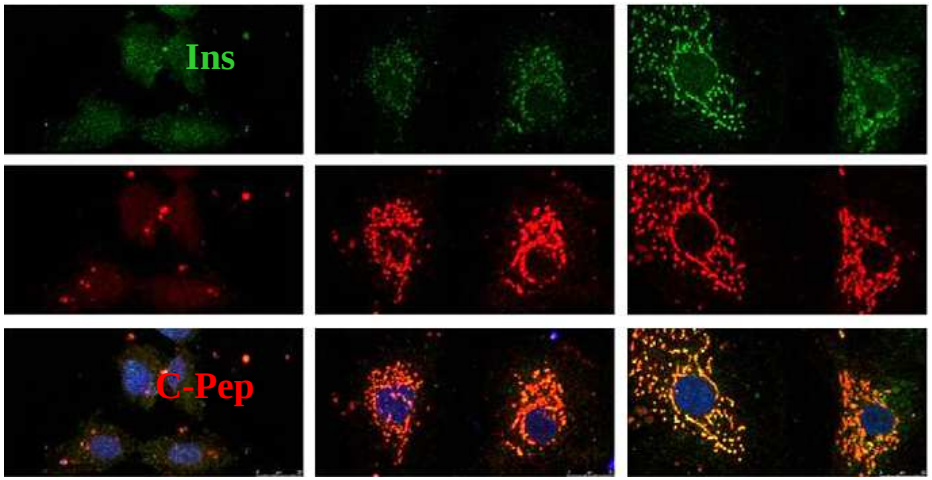
DMEM + SR 12%

Pancreatic commitment

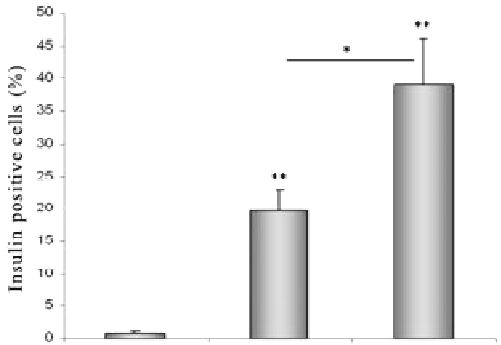
Suspension culture

DMEM-SR 12% + Fib/ITS

Factors - + +
Resveratrol - - +



Maturation



Factors - + +
Resveratrol - - +

Opinion

Toward cell-based therapy of type I diabetes

Dieter Kabelitz[1], Edward K. Geissler[2], Bernat Soria[3], Insa S. Schroeder[4], Fred Fändrich[5] and Lucienne Chatenoud[6]

Opinion

Trends in Immunology Vol.29 No.2

Trends in Immunology (2008) 29: 68-74

Stem Cell Review Series

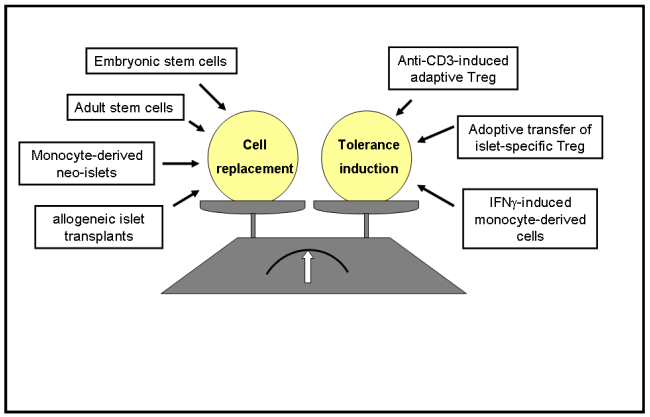
J. Cell. Mol. Med. Vol 13, No 8A, 2009 pp. 1464–1475

The immune boundaries for stem cell based therapies: problems and prospective solutions

Hmadcha Abdelkrim [a, *], Domínguez-Bendala Juan [b], Wakeman Jane [c], Arredouani Mohamed [d], Soria Bernat [a]

[a] Department of Cell Therapy and Regenerative Medicine, Andalusian Center for Molecular Biology and Regenerative Medicine (CABIMER), Sevilla, Spain
[b] Pancreatic Development & Stem Cell Laboratory, Diabetes Research Institute, University of Miami Leonard M. Miller School of Medicine, Miami, FL, USA
[c] North West Cancer Research Fund Institute, School of Biological Sciences, University of Wales Bangor, Memorial Building, Gwynedd, UK
[d] Department of Surgery, Beth Israel Deaconess Medical Center, Boston, MA, USA

Received: February 27, 2009; Accepted: June 25, 2009



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Immunomodulation

"Immunomodulatory Therapy": Clinical Trials

Target	Drug	Year(s)
CD3	Teplizuma, Otexizumab	2011
CD20	Rituximab	2009
CD80, CD86	Abatacept	2011
mTOR, IL-R2	IL-2, Rapamicin	2011
GAD65	GAD65-alum	2011
Insulin	Insulin	2011
HSP60	DiaPep277	2011
Cow's Milk Proteins	Cow´s milk (prevention)	2010

"Human vs NOD-mice Results": NOD treatment before insulitis…

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Immunomodulation with MSC

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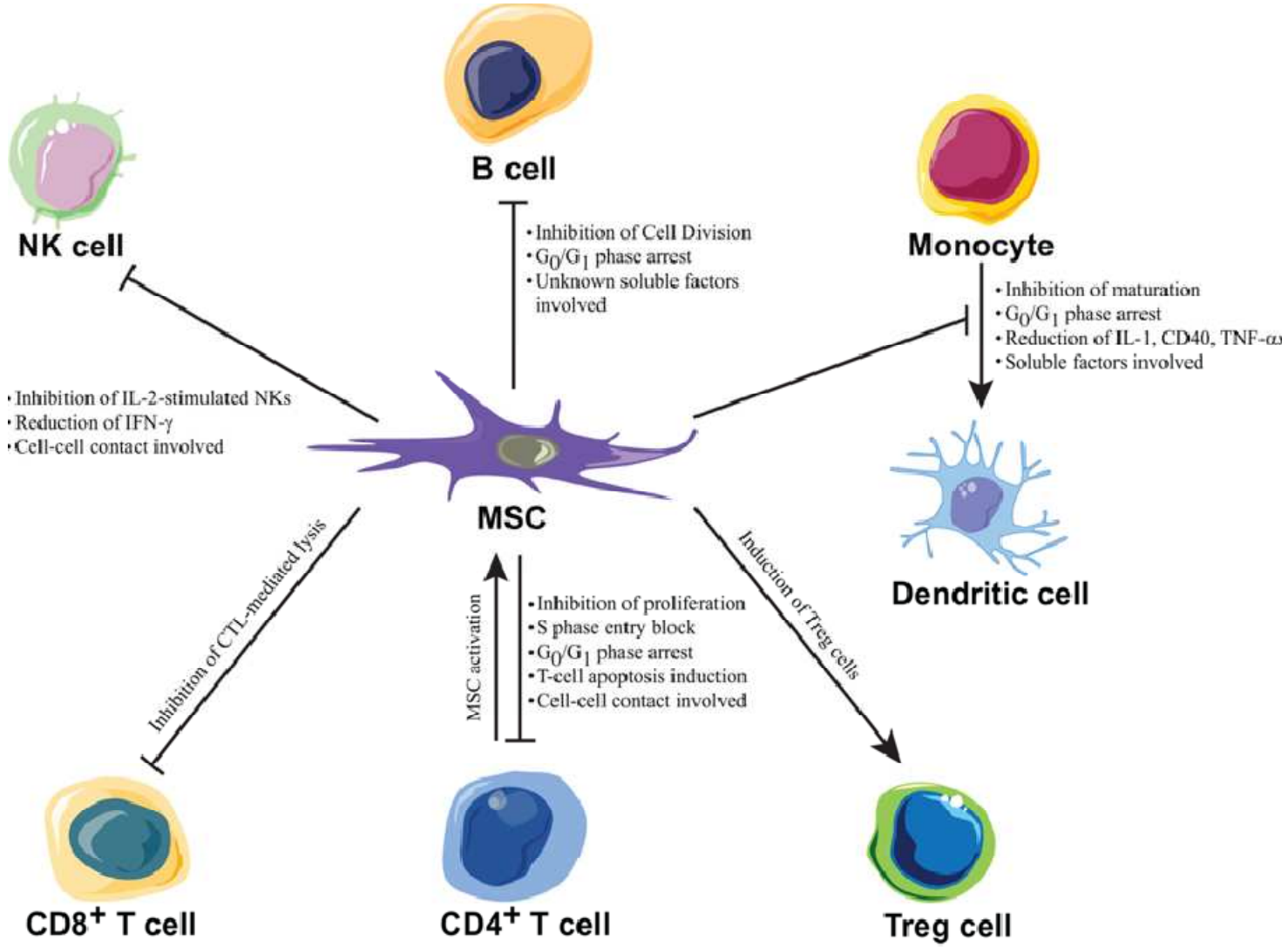
Mesenchymal stem cells are immunomodulators

1. Block T-cell proliferation,
2. Decrease TNFα and INF-γ
3. Increase IL-10 and IL-4
4. Up-regulate Treg
5. Inhibit NK cells
6. Inhibit Monocyte differentiation into DC cells. Immature DC cells more susceptible to degradation by NK cells, etc



Yi, Arch Pharm Res 35: 213-221, 2012

Child Autoimmune Diseases

- *Systemic Lupus Erythematosus*
- *Juvenile Idiopathic Arthritis*
- *Graft vs Host Disease*
- *Collagen-induced Arthritis*
- *Rheumatoid Arthritis*

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Cell Therapy of Liver Diseases

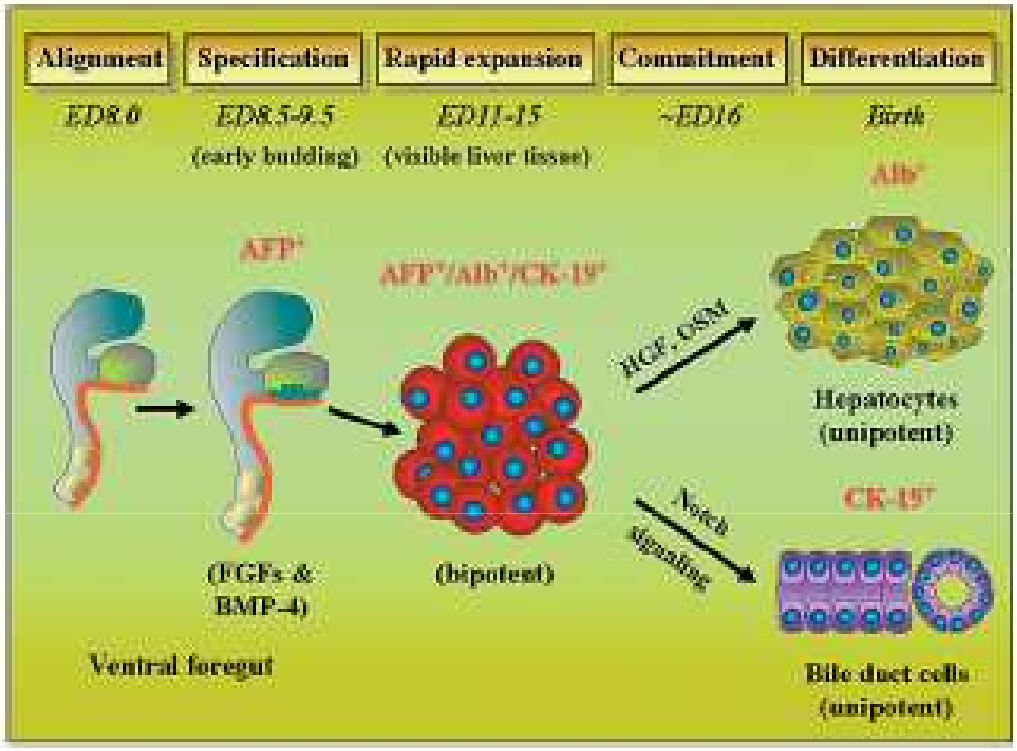

Fig. 2. Schematic representation of rodent fetal liver development.

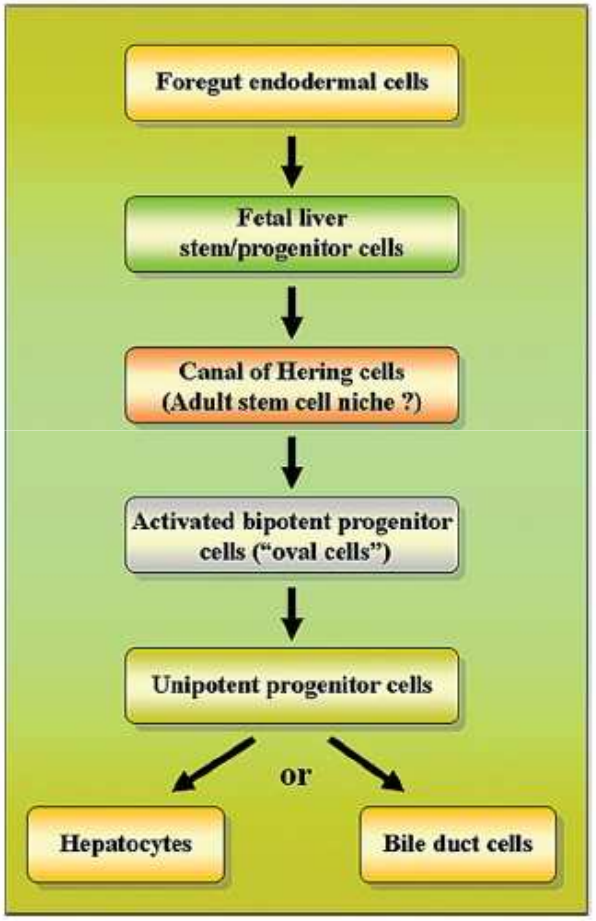

Fig. 3. Schematic diagram depicting liver epithelial cell lineage progression.

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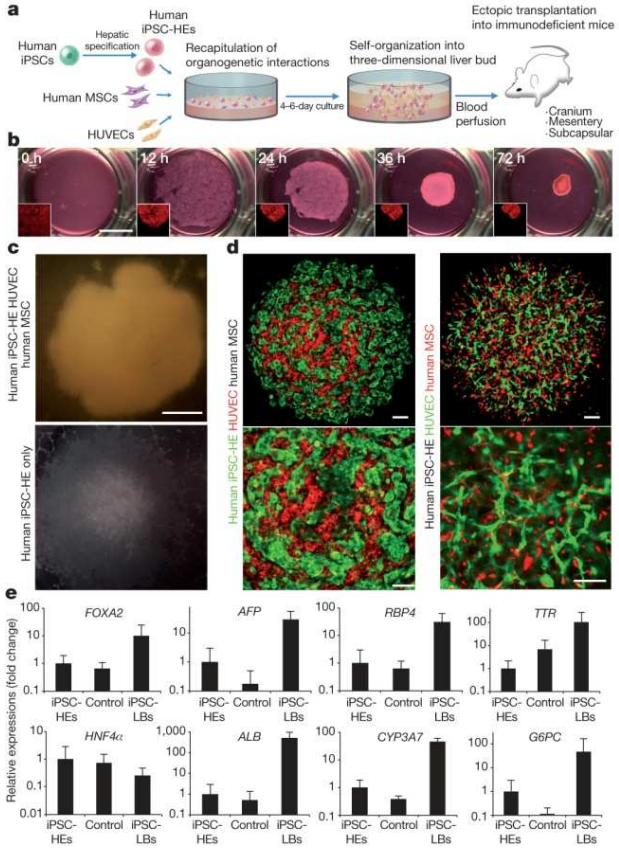
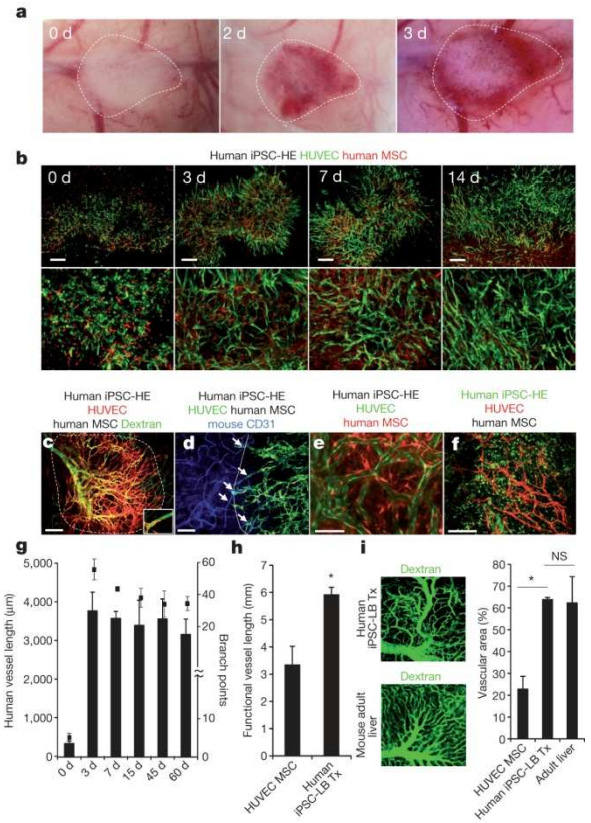
Vascularized and functional human liver from an iPSC-derived organ bud transplant

Takanori Takebe[1,2], Keisuke Sekine[1], Masahiro Enomura[1], Hiroyuki Koike[1], Masaki Kimura[1], Takunori Ogaeri[1], Ran-Ran Zhang[1], Yasuharu Ueno[1], Yun-Wen Zheng[1], Naoto Koike[1,3], Shinsuke Aoyama[4], Yasuhisa Adachi[4] & Hideki Taniguchi[1,2]

Nature **000**, 1-4 (2013)





Cell Therapy of Liver Diseases (1)

	Ref	Disease	Dose/Via	Results
BM-MNc (Autologous)				
	Terai, 2006	*Liver cirrosis*	*5 x 10^9*	*Safe* *↑Albumin*
	Lyra, 2007	*End Stage Chronic Liver Disease*	*100 x 10^6* *Hepatic artery*	*Safe, Feasible* *↑Albumin* *↓Bilirrubin*
	Lyra 2010	*Hepatic Cirrosis*	*Hepatic artery*	*↑Albumin* *↓Bilirrubin*
CD 133+ (Autologous)				
	Fürst, 2007	*PVE+Hepatectomy*		*↑ Regeneration*
	Salama 2010	*End-stage liver disease*	*CD34+/CD133+* *(G-CSF mobil)*	*Improve liver function* *↑Liver enzymes*
	Nikeghbalian, 2011	*Cirrosis*	*CD133+ vs BM-MNc*	*No adverse effects*
	Esch, 2012	*Hepatectomy*		*↑ Proliferation*
	Lehwald, 2013		*CD133* *(HGF/SDF1 mobil)*	

Cell Therapy of Liver Diseases (2)

	Ref	Disease	Dose/Via	Results
CD34+ (Autologous)				
	Gordon, 2006	*Chronic Liver Disease*	*10^6 -2 x 10^8 Portal vein/ Hepatic artery*	*Safe ↑Albumin ↓Bilirrubin*
	Levicar, 2008	*Chronic Liver Disease*	*10^6 -2 x 10^8 Portal vein/ Hepatic artery*	*Safe*
	Pai, 2008	*Alcoholic cirrosis (Chronic Liver disease)*	*Expanded CD34+*	*Improvement Bilirrubin & Liver enzymes*
	Garg, 2012	*Acute on Chronic Liver Failure*	*G-CSF*	*↑ Survival*
Preclinical				
	Mintz, 2013	*Animal Model NOD-SCID fibrosis*	*CD34+ conditioned medium*	*Improvement*

Cell Therapy of Liver Diseases (3)

	Ref	Disease	Dose/Via	Results
BM-MSC (autologous)				
	Mohamadnejad, 2007	*Liver cirrosis*	*3 x 10^7*	*Improved MELD*
	Kazahira, 2009	*Liver cirrosis*	*3,5 x 10^7*	*Improved MELD, serum creatinine, prothrombin complex*
	Amer,	*End stage liver failure*	*2 x 10^7 cells (pretreated HGF: liver committed)*	*Improved MELD*
Preclinical				
	Tanimoto, 2012	*Murine Liver cirrosis*	*5 x 10^5*	*↓ Liver Fibrosis ↑ MMP-9; ↓ αSMA, ↓TNFα, ↓TGFβ*
LIVER CELLS (Heterologous)				
Progenitors (MSC-like)	***PROMETHERA (Belgium) Sokal,***	***Crigler-Najjar Urea Cycle deficiencies***	***HepaStem (adult liver progenitor cells)***	
Hepatocytes (isolated/cultured)	***Castell, 2103***	***Inherited Liver dis (Crigler-Najjar,) Liver failure etc***	***Hepatocyte (from donor livers)***	

Conclusiones:

EFECTOS BENEFICIOSOS, pero

- *Muchos Ensayos Fase I-II*
- *No ensayos Fase II y Fase III*
- *La mayoría no se publican*
- *Mecanismo desconocido*
- *No hay una hipótesis directriz*

- *Muchos Ensayos Fase I-II*

Familia de factores de transcripción GATA

- *Están muy conservados en distintas especies*
- *Se unen a la secuencia GATA de los promotores de los genes a a los que activa*

GATA 1, 2, 3 → Hematopoiesis

GATA 4, 5, 6 → Se expresan en:

- EMBRIÓN: mesodermo y endodermo
- ADULTO: corazón, pulmones, hígado y páncreas

Mutaciones en genes GATA asociados a defectos cardíacos en humanos

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American Journal of Medical Genetics 83:201–206 (1999)

GATA4 Haploinsufficiency in Patients With Interstitial Deletion of Chromosome Region 8p23.1 and Congenital Heart Disease

Tugce Pehlivan,[1] Barbara R. Pober,[2,3] Martina Brueckner,[2] Stacey Garrett,[4] Rachel Slaugh,[4] Richard Van Rheeden,[4] David B. Wilson,[4,5]* Michael S. Watson,[4,6] and Anne V. Hing[4]

HUMAN MUTATION Mutation in Brief #881 (2006) Online

letters to nature

GATA4 mutations cause human congenital heart defects and reveal an interaction with TBX5

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MUTATION IN BRIEF

A Novel Mutation in the *GATA4* Gene in Patients With Tetralogy of Fallot

Georges Nemer[1,*], Fatimah Fadlalah[1], Julnar Usta[1], Mona Nemer[2], Ghassan Dbaibo[1], Mounir Obeid[3], and Fadi Bitar[3]

PNAS

GATA6 mutations cause human cardiac outflow tract defects by disrupting semaphorin-plexin signaling

Kazuki Kodo[a,1], Tsutomu Nishizawa[b], Michiko Furutani[b,c], Shoichi Arai[b], Eiji Yamamura[c], Kunitaka Joo[d], Takao Takahashi[a], Rumiko Matsuoka[b,c,e,1], and Hiroyuki Yamagishi[a,b,1]

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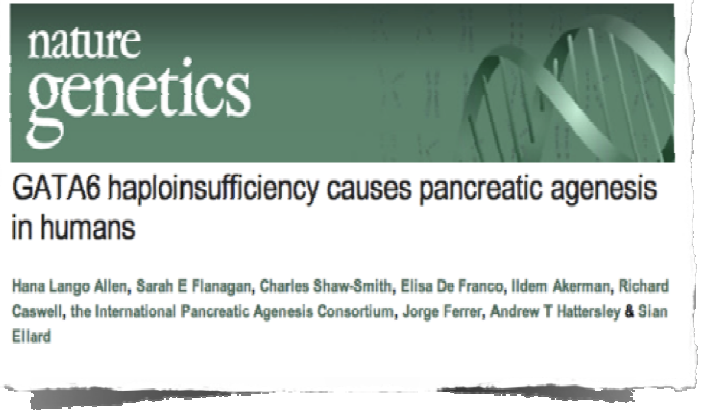
Edited by Eric N. Olson, University of Texas Southwestern Medical Center, Dallas, TX, and approved July 1, 2009 (received for review April 30, 2009)

Journal of Human Genetics **55**, 662–667 (October 2010) | doi:10.1038/jhg.2010.

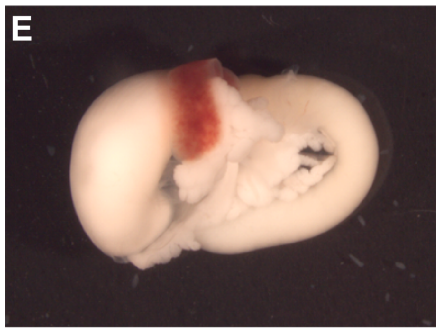
A novel GATA6 mutation in patients with tetralogy of Fallot or atrial septal defect

Xiaoping Lin, Zhaoxia Huo, Xingyuan Liu, Yangyang Zhang, Li Li, Hong Zhao, Biao Yan, Ying Liu, Yiqing Yang and Yi-Han Chen

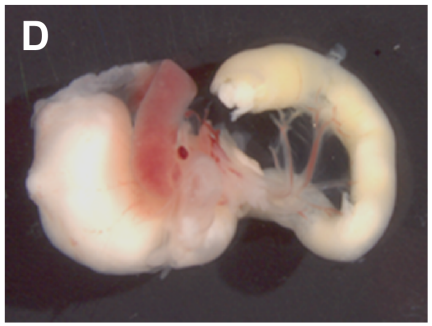
Función de los factores GATA en la formación del páncreas



Control



GATA4/GATA6 KO



Factores GATA

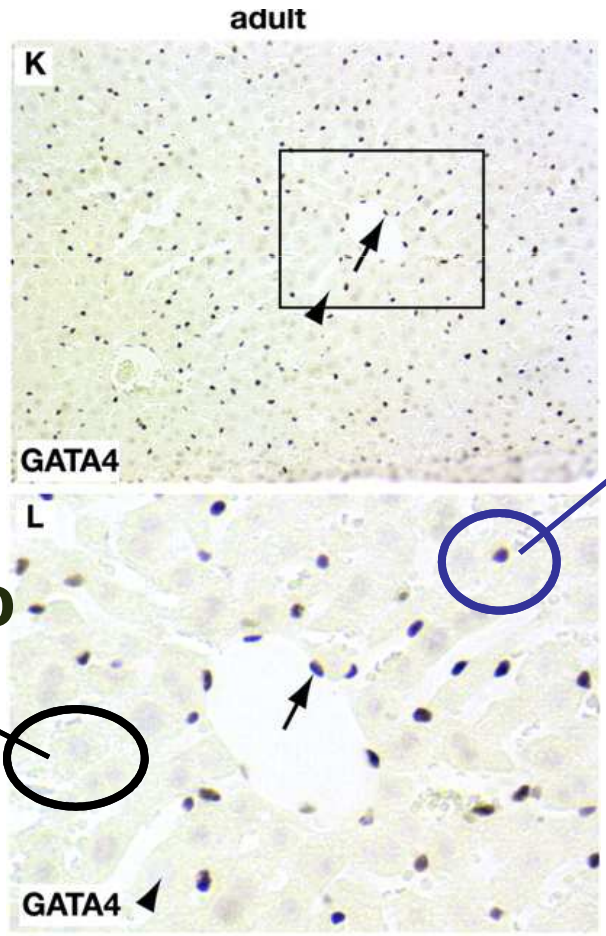
Progenitores pancreáticos

Proliferación

Diferenciación

Identidad

GATA4 **se expresa en las**
células estrelladas hepáticas (mesodermo),
pero no en los hepatocitos (endodermo)

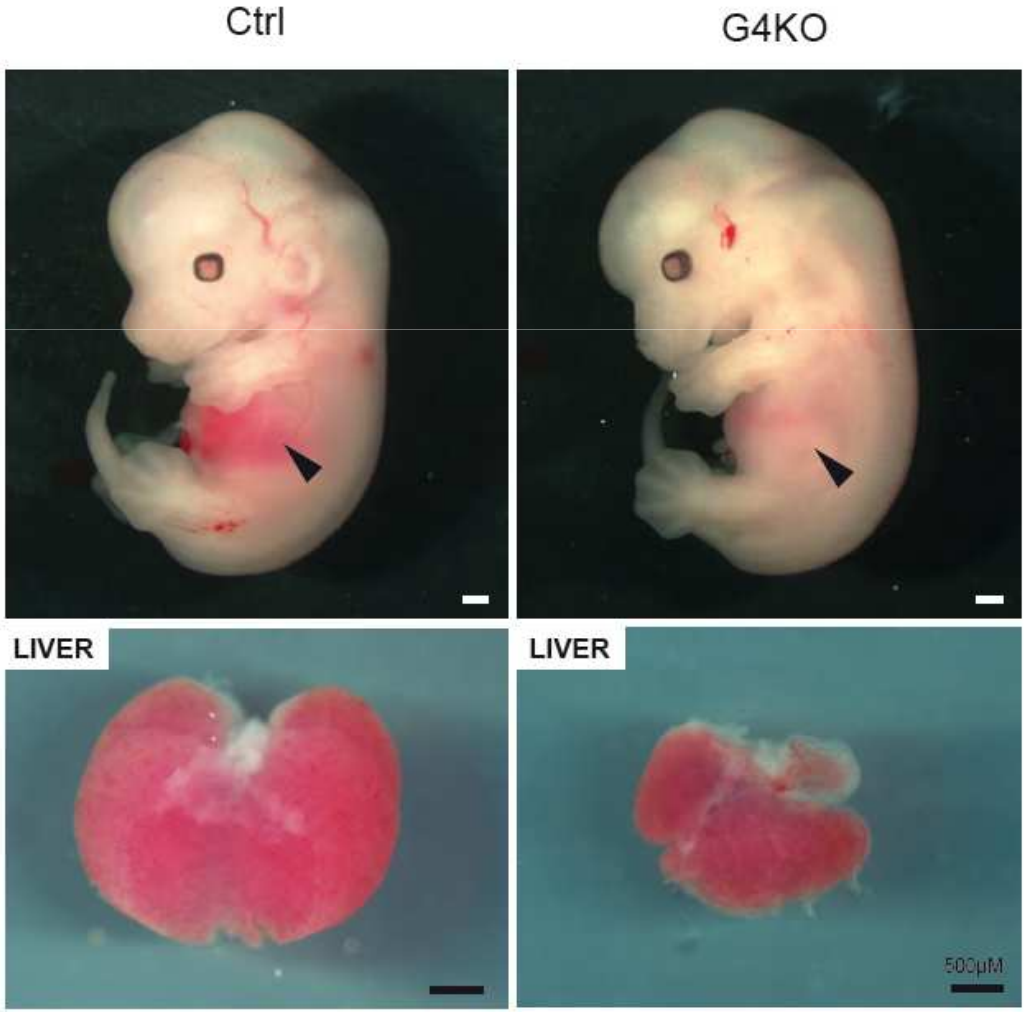


Célula hepática estrellada

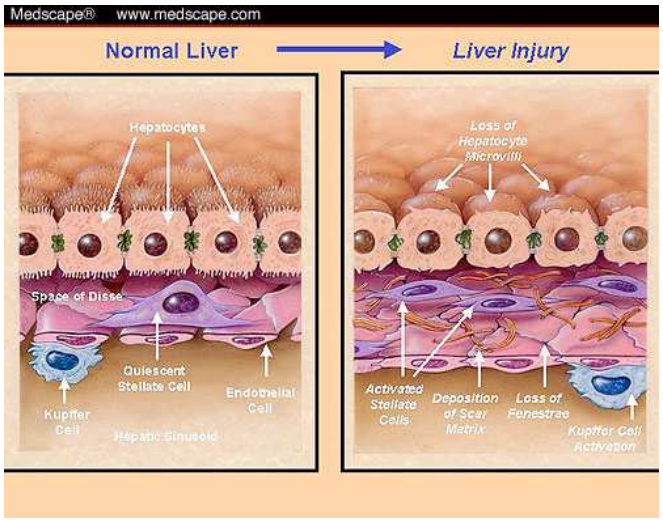
Hepatocito

Inactivación de GATA4

(específicamente) en las células estrelladas del hígado produce hígado hipoplásico y cirrótico



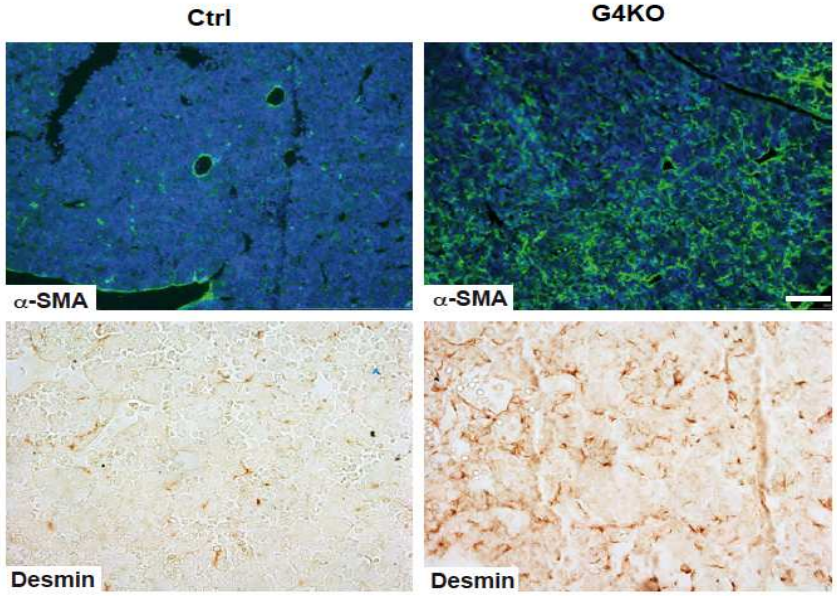
GATA4 -/-
Hígado
hipoplasico y cirrótico



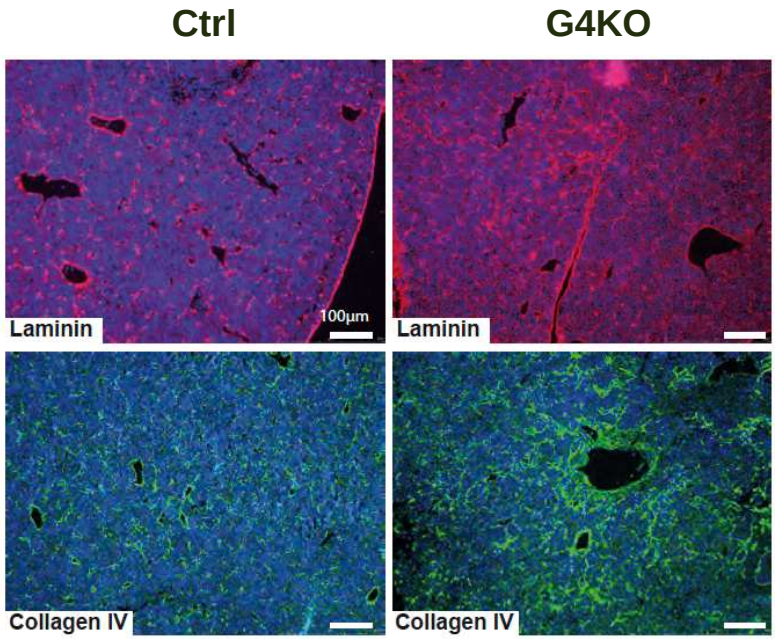
Ausencia de GATA4:

1. *Activación de células estrelladas hepáticas*
2. *Deposición de proteínas de matriz extracelular (colágeno IV, laminina)*

Activación de Células EstrelladasHSCs



Deposición de proteínas de matriz extracelular



Los niveles de expresión de GATA4 en células estrelladas humanas disminuye durante la fibrosis y cirrosis

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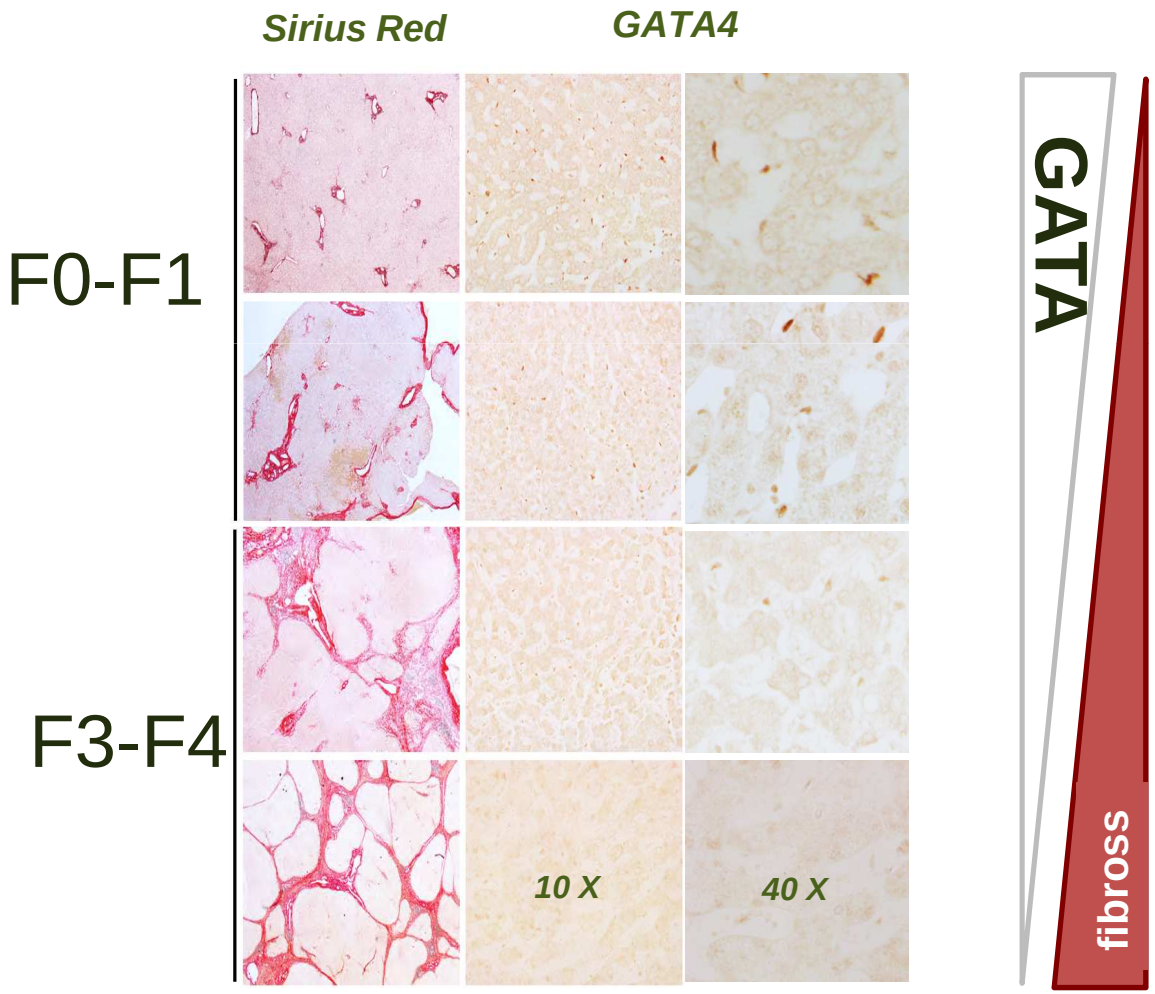
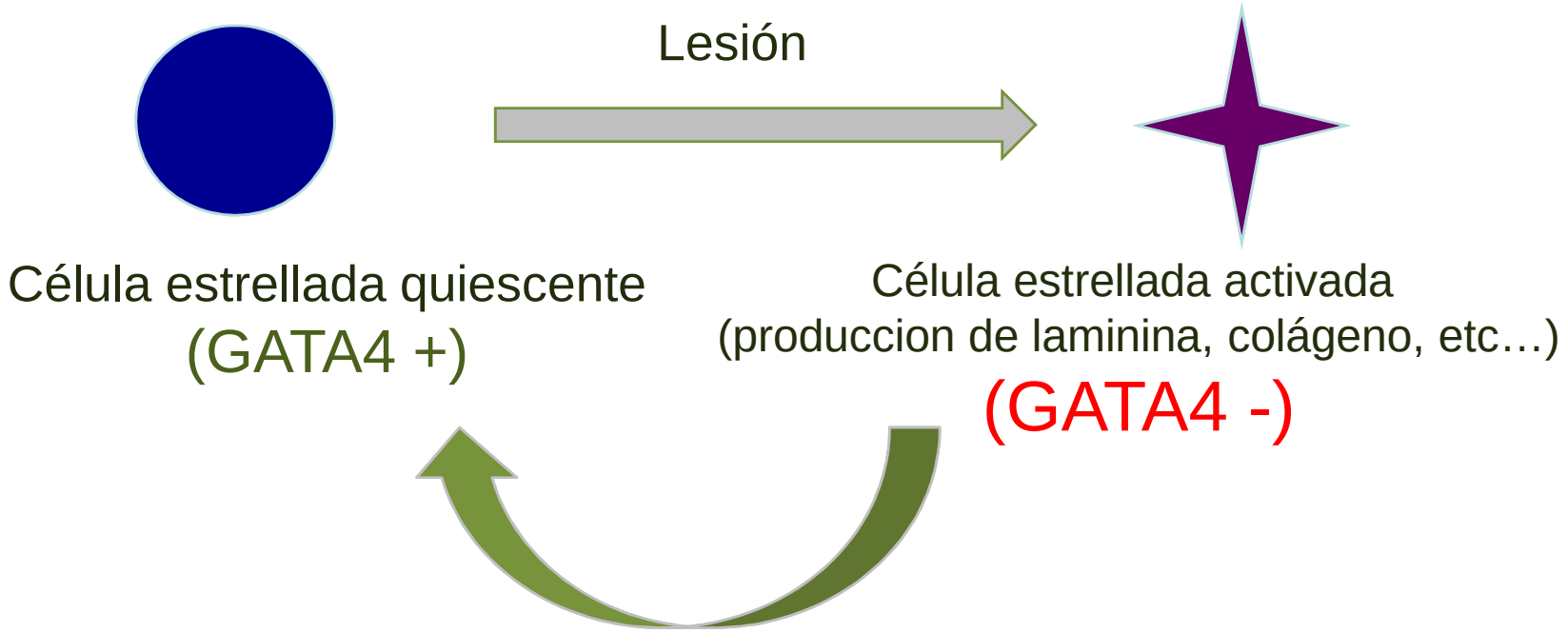
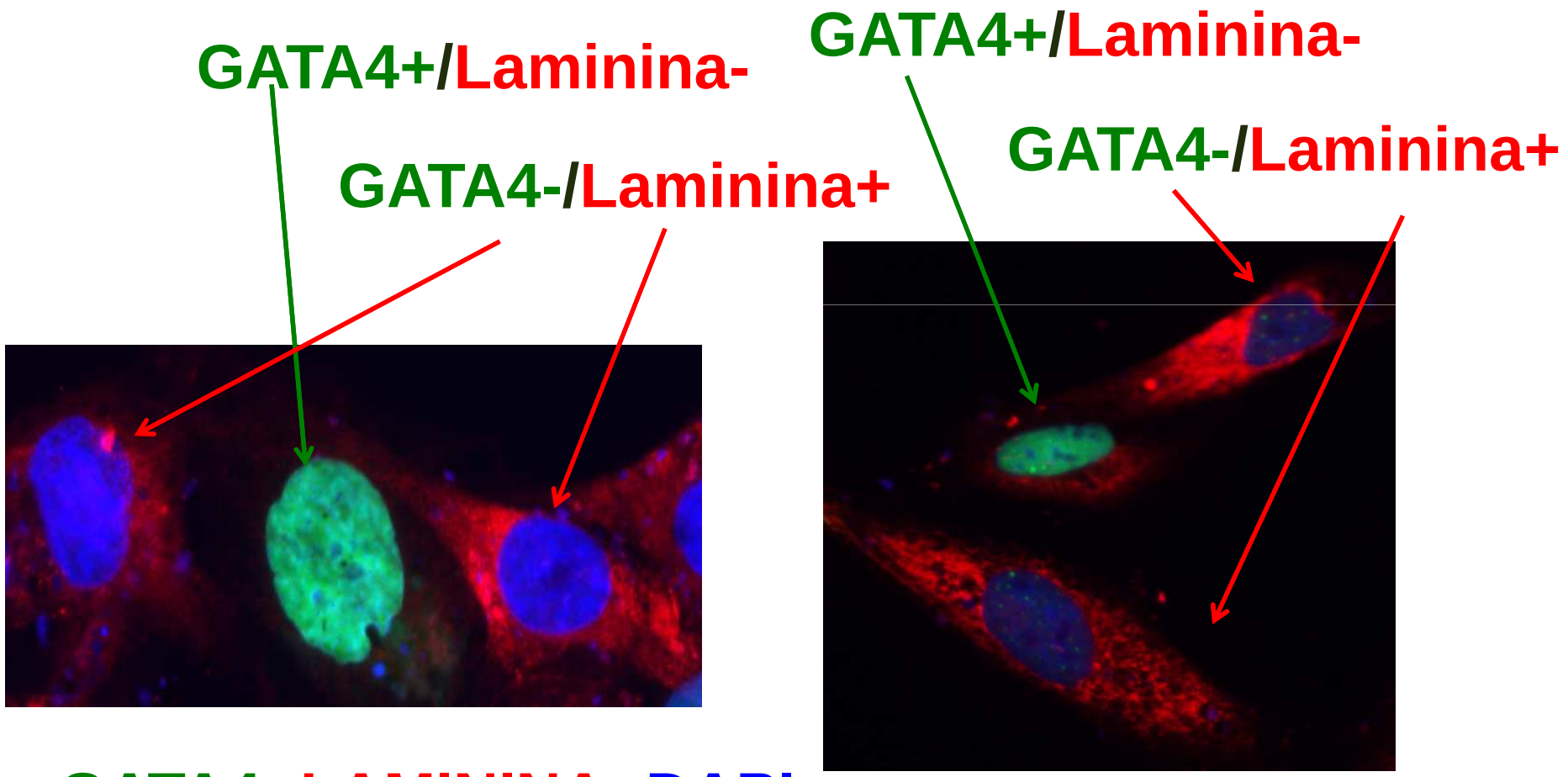


La inducción de la expresión de GATA4 en células estrelladas activadas revierte o atenúa la progresión de la fibrosis?



Sobreexpresión de GATA4 en línea célular estrellada humana (activada) disminuye/revierte el fenotipo activado



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¿ Como podemos sobreexpresar GATA4 en la Célula Estrellada Humana

para revertir el fenotipo activado (fibrosis-cirrosis?

BUSQUEDA DE COLABORACIÓN:

¿ Tienen algun caso de

- *Fibrosis Neonatal asociada a un cuadro.*
  - *pancreático?*
  - *cardíaco?*

1. *Biopsia hepática y GATA 4*
2. *Posible aproximación terapéutica para la disminución de la Fibrosis*

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Osteogenesis Imperfecta

The meaning, the sense and the significance: translating the science of mesenchymal stem cells into medicine

Paolo Bianco[1], Xu Cao[2], Paul S Frenette[3], Jeremy J Mao[4], Pamela G Robey[5], Paul J Simmons[6] & Cun-Yu Wang[7]

NATURE MEDICINE VOLUME 19 | NUMBER 1 | JANUARY 2013

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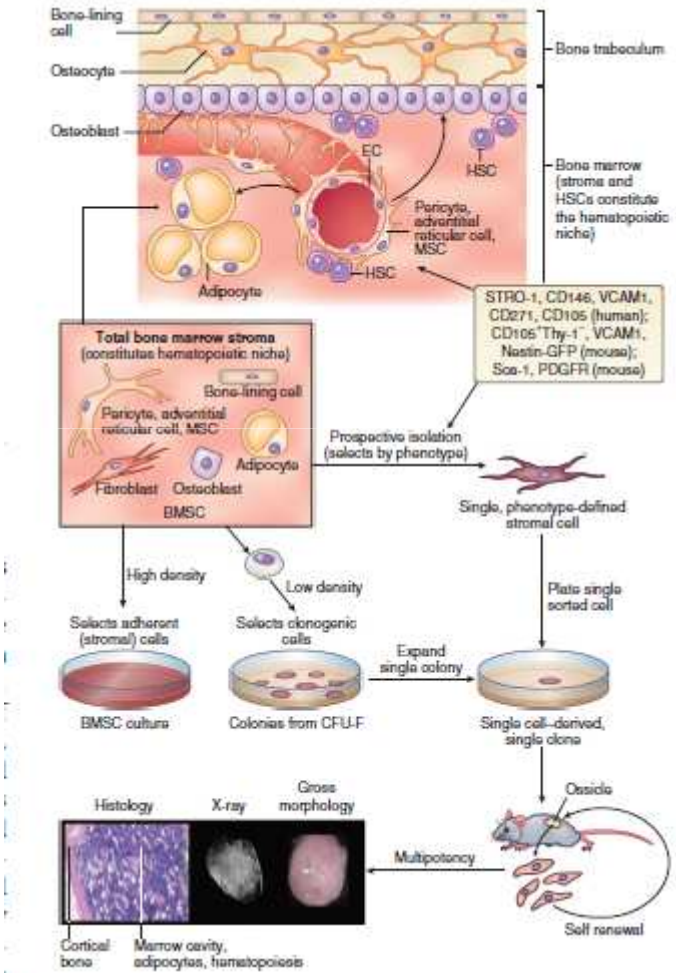

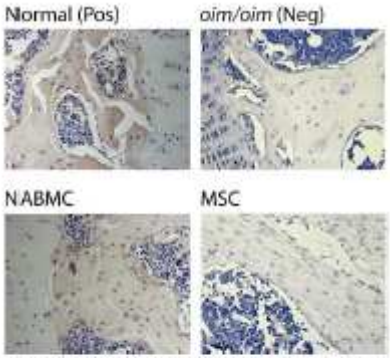

Figure 3. Photomicrographs of bone from oim/oim mice after cell infusions. Mice were infused with either NABMC or MSCs from wild-type mice and then the bones were immunostained with a polyclonal antibody which recognizes only the pro α2 polypeptide (not pro α1) and visualized with NOVARed. The positive control (top left panel) is normal mouse bone which demonstrates staining of the trabecular bone but not the growth-plate cartilage on the right side of the section. The negative control (top right panel) is oim/oim mouse bone, which does not express pro α2 peptide. Oim/oim mice infused with NABMC (bottom left panel) show red stain (pro α2 expression) in the trabecular bone but not the articular cartilage on the left side of the section. Oim/oim mice infused with MSCs (bottom right panel) lack any red staining indicating the lack of detectable pro α2. Original magnification, ×200.

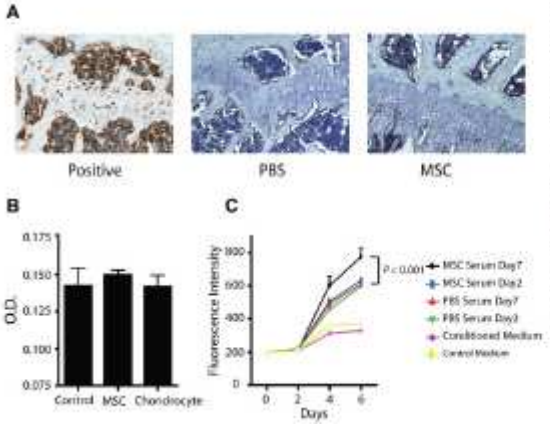

Figure 4. The effect of MSCs on chondrocyte proliferation. (A) GFP staining of the growth plate after GFP-positive MSC infusion. Immunostaining for GFP expression of the growth plate from a GFP-transgenic mouse (positive), a mouse after saline infusion (PBS, negative), or a mouse after GFP-transgenic MSC infusion. Original magnification, ×200. (B) Chondrocyte proliferation was analyzed by MTT Cell Proliferation assay after 3-day coculture with MSCs or chondrocytes or control medium on the transwell plates ($n = 3$). (C) Sera were collected from MSC-injected and PBS-injected mice 2 days and 7 days after the injection. Chondrocyte proliferation assay was performed at day 0, day 2, day 4, and day 6 after culture with the sera. MSC-conditioned medium, and control medium ($P < .001$, $n = 6$). The depicted data are a representative experiment. In total, 38 groups of mice (202 experimental, 154 controls) confirmed these findings. (D) Sera from mice injected with control medium, MSC-conditioned medium, or MSCs were applied into chondrocyte culture. Chondrocytes were cultured for 6 days in serum-supplemented medium followed by the proliferation assay measured as fluorescence intensity ($^*P < .05$, $n = 9$). All data are mean ± SEM.

Transplanted bone marrow mononuclear cells and MSCs impart clinical benefit to children with osteogenesis imperfecta through different mechanisms

Satoru Otsuru,[1] Patricia L. Gordon,[2] Kengo Shimono,[3] Reena Jethva,[1] Roberta Marino,[1,2] Charlotte L. Phillips,[4] Ted J. Hofmann,[1,2] Elena Veronesi,[5] Massimo Dominici,[5] Masahiro Iwamoto,[3] and Edwin M. Horwitz[1,2]

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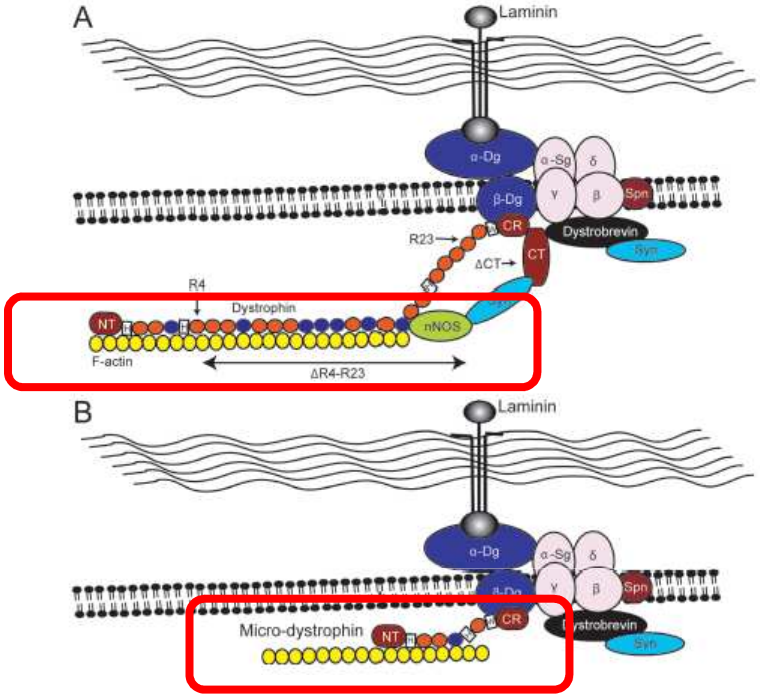
Muscular Dystrophies



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CELL THERAPY APPROACHES

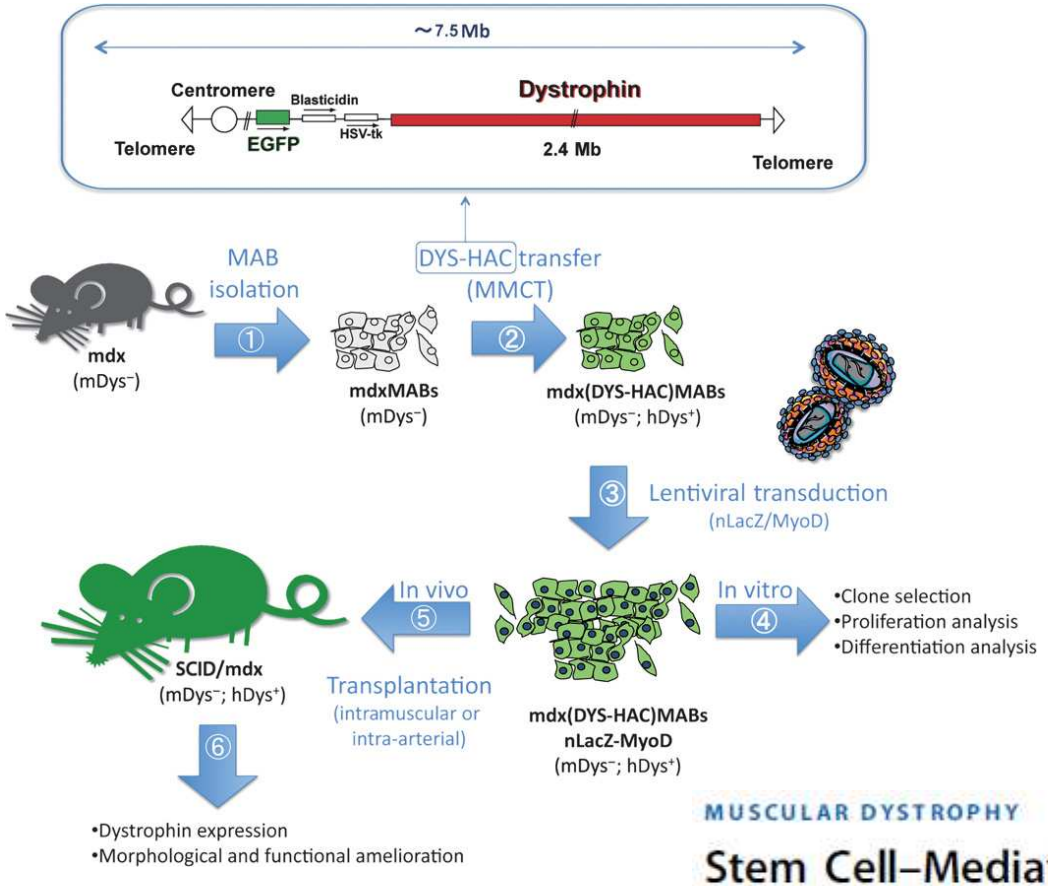
- *BM-MNc (CTrial)*
- *Fetal Skeletal Muscle Progenitors*
- *Mesoangioblasts (CTrial)*

- *Fibrosis: MSC (matrix metalloproteinase)*

Neurological and Neurodegenerative Disorders

1. *Lisosomal Storage Diseases: Phase I-IIa (Safe/Feasible)*
   - *Neuronal Lipofuscinosis (HuNSC + Immunosuppression J Neurosurg Pediat 11(6) 643-652)*
2. *Cerebral Paralsy*
   - *Cord Blood (less immunogenic)*
3. *Etc*

Futuro



MUSCULAR DYSTROPHY

Stem Cell–Mediated Transfer of a Human Artificial Chromosome Ameliorates Muscular Dystrophy

www.ScienceTranslationalMedicine.org 17 August 2011 Vol 3 Issue 96 96ra78

Laboratorio
Terapia Celular
DM
UPO Marzo 2004

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