“open up your mind let your fantasies unwind.”

QUESTIONS?

Programma

Corso di Laurea in Comunicazione scientifica biomedica

Processi comunicativi scienza e medicina modulo I
Isabella Saggio

Prerequisiti
Conoscenze di base di genetica e biologia cellulare

Obiettivi
Fornire agli studenti le conoscenze di base relative alle cellule staminali, alla genetica del tumore, agli organismi geneticamente modificati per portare studenti e studentesse non solo ad un miglioramento delle conoscenze ma anche alla capacità critica nella valutazione di dati sperimentali e clinici e alla loro corretta comunicazione-

Conoscenze e comprensione
Genetica, biomedicina e biologia delle cellule staminali

Capacità di applicare conoscenze e comprensione
Genetica e medicina molecolare

Capacità critiche e di giudizio
Valutazione dei punti forti e deboli della genetica e della medicina traslazionale

Capacità di comunicare quanto appreso
Discussione di gruppo dei temi del corso e comunicazione

Capacità di proseguire lo studio in modo autonomo nel corso della vita
Maturazione di capacità critica e di comunicazione oltre che della comprensione della letteratura della genetica e della medicina traslazionale

Programma
Il corso intende approfondire i processi comunicativi della scienza e della medicina, con una prospettiva sia clinica che biologica. Si intende fornire le conoscenze relative ai fondamenti della genetica di base, della genetica del cancro, del trasferimento genico, dei sistemi di correzione genetica (e.g. CrisprCas9). Oltre che conoscenze sui processi aberranti nelle malattie genetiche. Le cellule staminali embrionali, adulte e indotte verranno discusse in relazione a specifiche patologie genetiche e acquisite, fra cui la fibrosi cistica, la distrofia muscolare, le displasie ossee, le immunodeficienze, le progerie, il cancro. Verranno discussi i dati preclinici in modelli animale e clinici in uomo anche con una prospettiva della comunicazione della scienza e della medicina con i media.
Il corso prevede una parte pratica di comunicazione della scienza e della medicina per lo sviluppo di una comunicazione efficace e critica della ricerca di base e applicata.

Testi adottati e bibliografia di riferimento
- Hartwell et al.: GENETICA dall'analisi formale alla genomica
- Saggio: L'età se esiste
- Bencivelli de Ceglia: Comunicare la scienza
- Meldolesi: E l'uomo creò l'uomo. CRISPR e la rivoluzione dell'editing genomico

Il materiale didattico è disponibile sulla piattaforma e-learning

Modalità di svolgimento
Lezioni frontali e laboratori di scrittura su argomenti specifici trattati nel corso

Modalità di valutazione
Per i e le frequentanti (70% lezioni), la prova di esame sarà la redazione di pezzi di comunicazione della scienza e della medicina con tutoraggio in aula. Per i e le non frequentanti la prova di esame sarà la redazione di un articolo in classe senza fonti, in data di appello. Per tutti la verbalizzazione sarà in data di appello.

Lezioni

- Teoriche
- Pratiche
- Attività integrative

References:

- Hartwell et al.: GENETICA dall'analisi formale alla genomica
- Alberts and Johnson: Molecular Biology of the Cell

- Saggio L’età se esiste
- Bencivelli de Ceglia Comunicare la scienza
- Meldolesi E l'uomo creò l'uomo. CRISPR e la rivoluzione dell’editing genomico

- Ppt via elearning

www.saggiolab.com

Esame

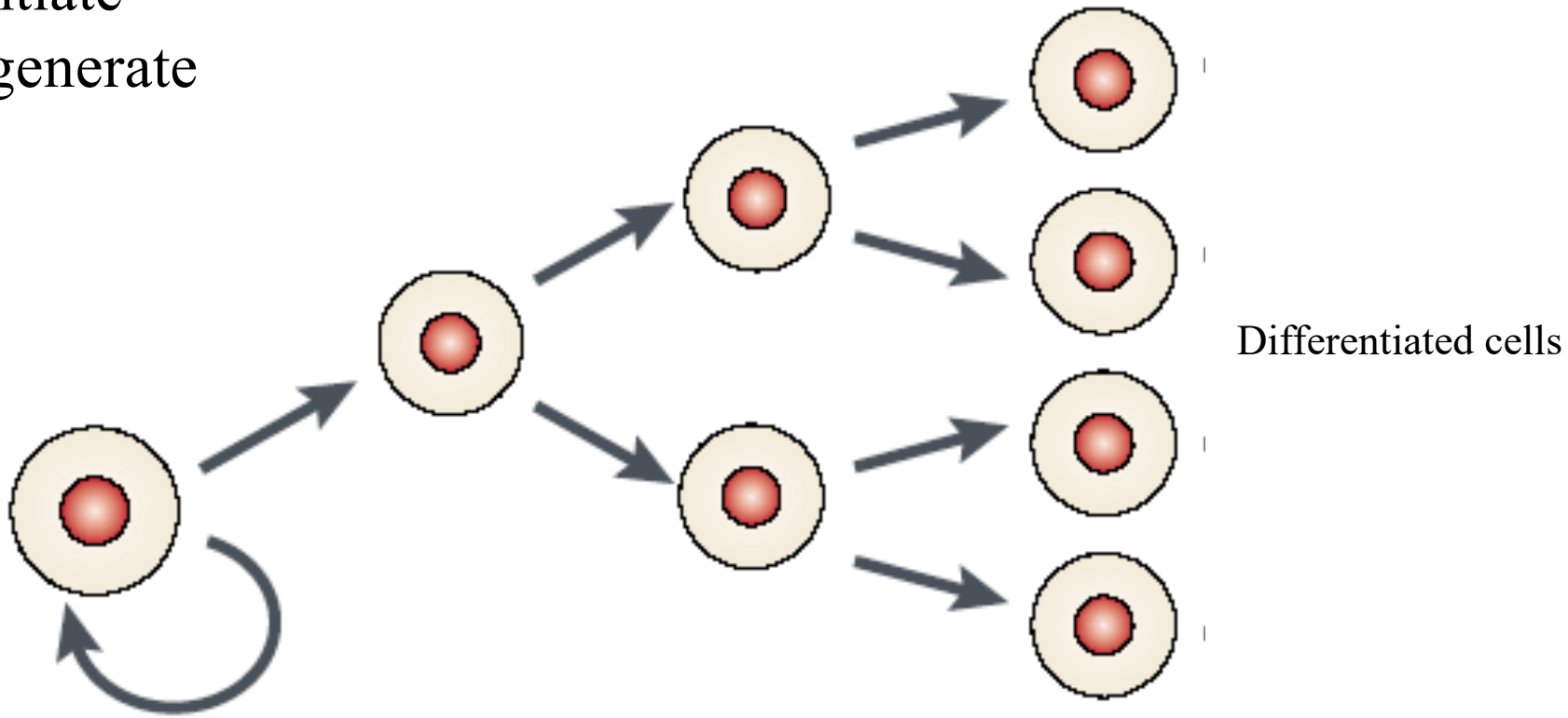
Modalità di valutazione

- Per i e le frequentanti (70% lezioni), la prova di esame sarà la redazione di pezzi di comunicazione della scienza e della medicina con tutoraggio in aula.
- Per i e le non frequentanti la prova di esame sarà la redazione di un articolo in classe senza fonti, in data di appello. Per tutti la verbalizzazione sarà in data di appello.


Svizzera
Lausanne
Lyon
Genève
Grenob
tienne
Valence
Gap
Montpellier
Monaco
Marseille
Toulon
Perpignan
Girona
arcelona
Ajaccio
Sassari
Cagliari
Modena
Bologna
Ravenna
Ancona
Roma
Foggia
Bari
Salerno
Taranto
Lecce
Tyrrhenian Sea
Catanzaro
Palermo
Messina
Catania
Siracusa
Ragusa
Gouvernorat de Tunis
Slovenija
Slovenia
Zagreb
Hrvatska
Croatia
Hungary
Pécs
Timişoara
Arad
Osijek
Beograd
(Београд)
Bosna i Hercegovina
Bosnia and Herzegovina
Split
Србија
Serbia
Crna Gora
Montenegro
Kosova
Kosovo
Shkodër
Shqipëria
Albania
Macedonia

Stem cells properties

1 Differentiate
2. Self regenerate



Asymmetric division

Symmetric division

History

~1900 self regenerating cell

1961 hemopoietic stem cell

1968 skeletal stem cells

1983 mouse ES

1997 Dolly the sheep (nuclear transfer)

1998 human ES

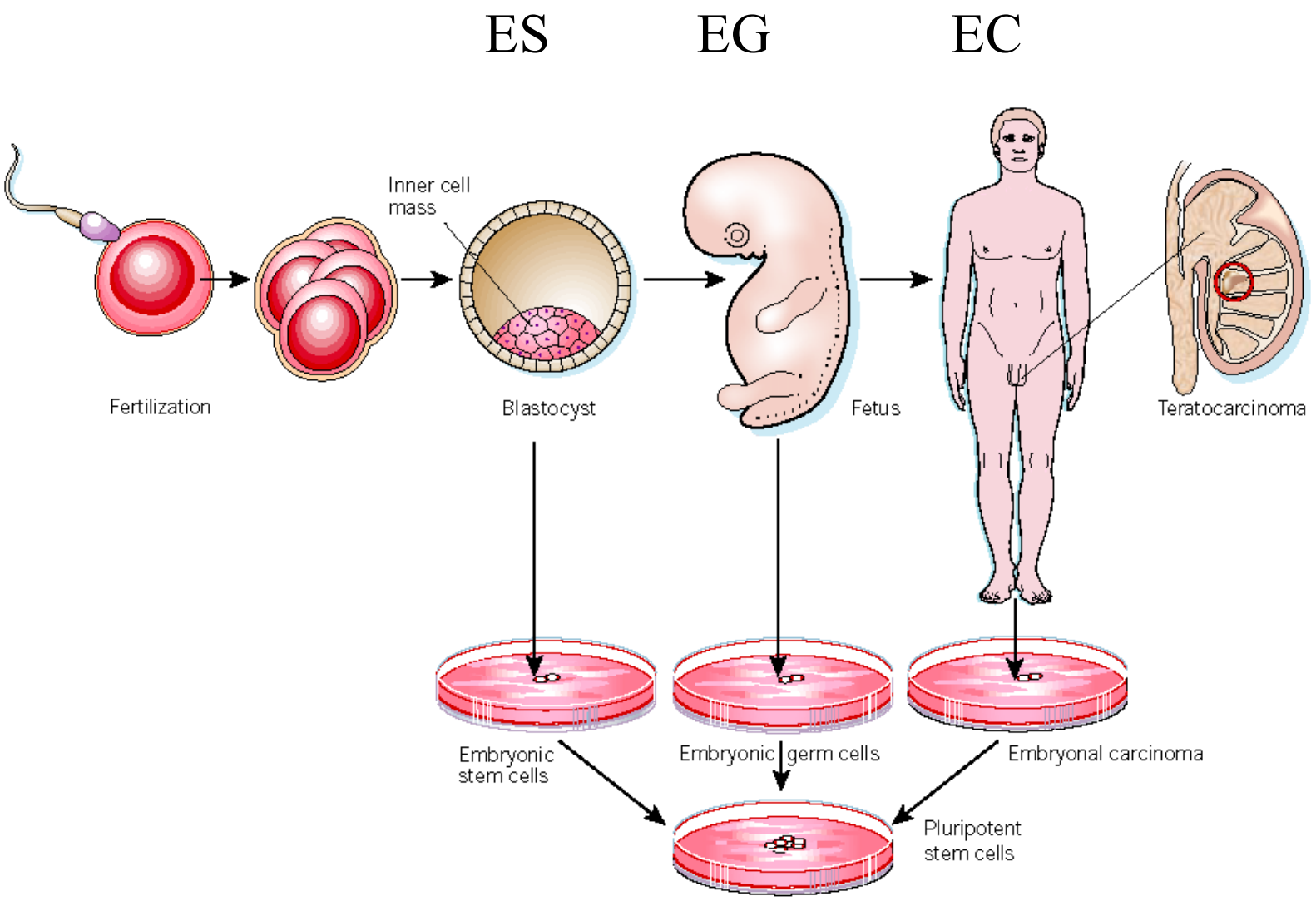
1999 adult stem cell

2006 IPS

Categories

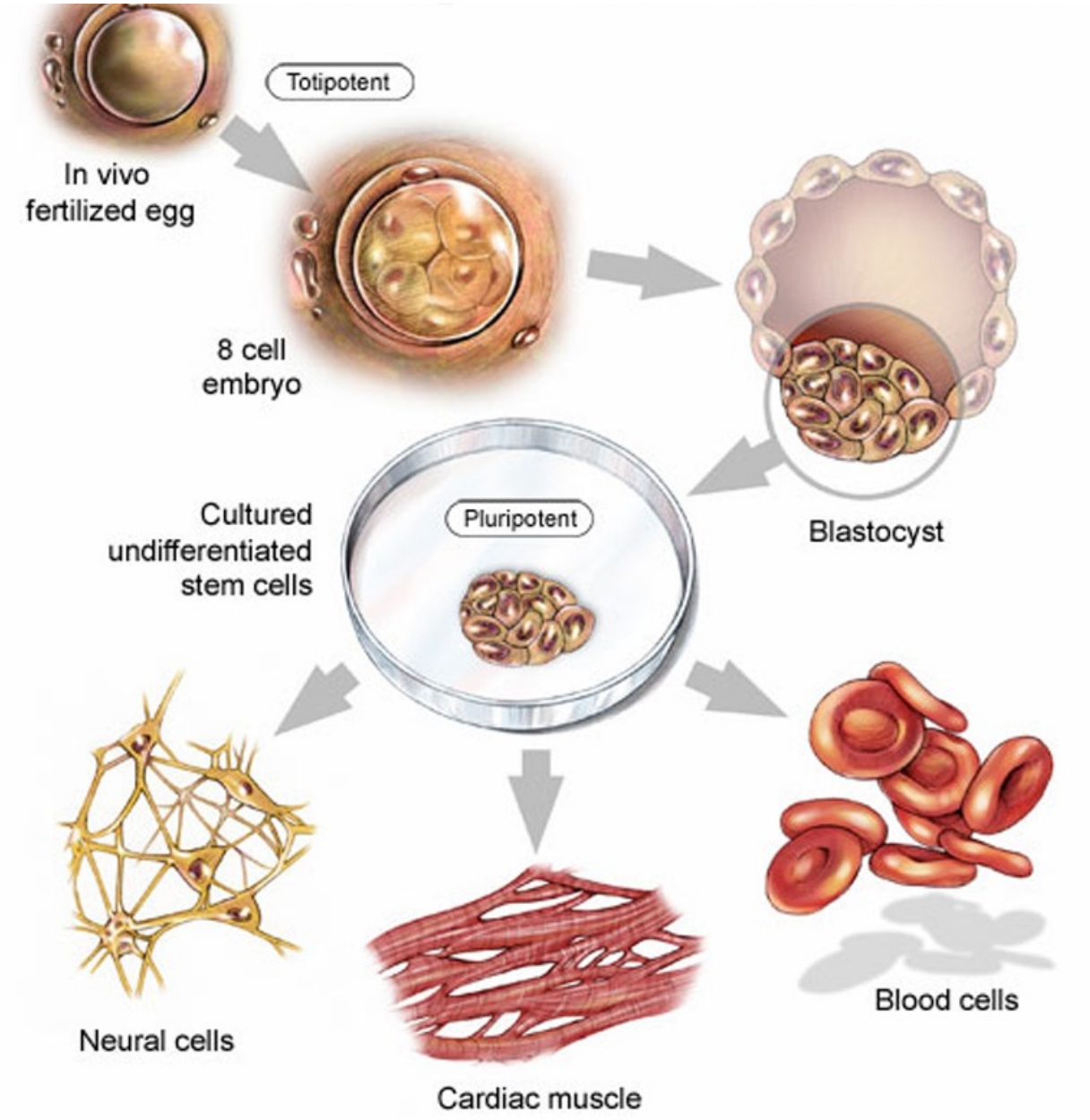
embryonic	ES
↓	EG (germ)
pluripotent	EC (carcinoma)
post-natal	hemopoietic
↓	epithelial
multipotent	skeletal
unipotent	……

ES origin

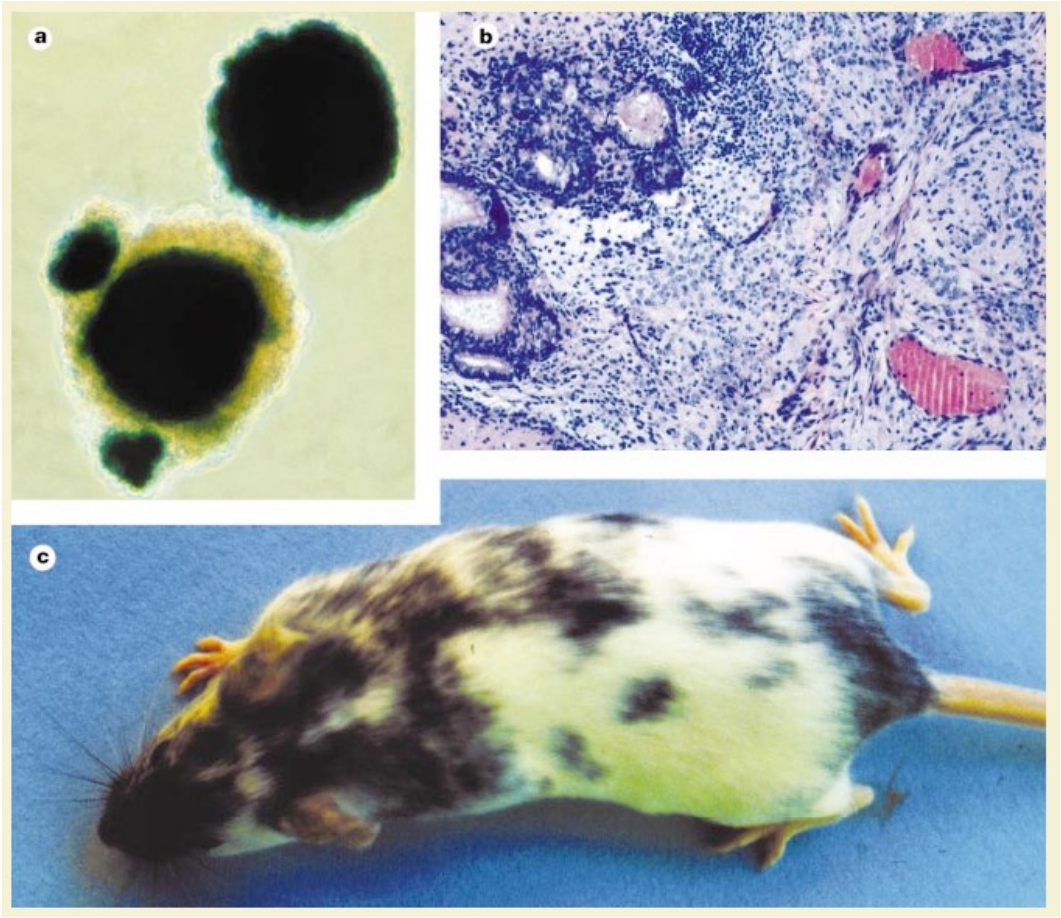


Teratocarcinoma: germ cell tumor

ES: in vitro differentiation



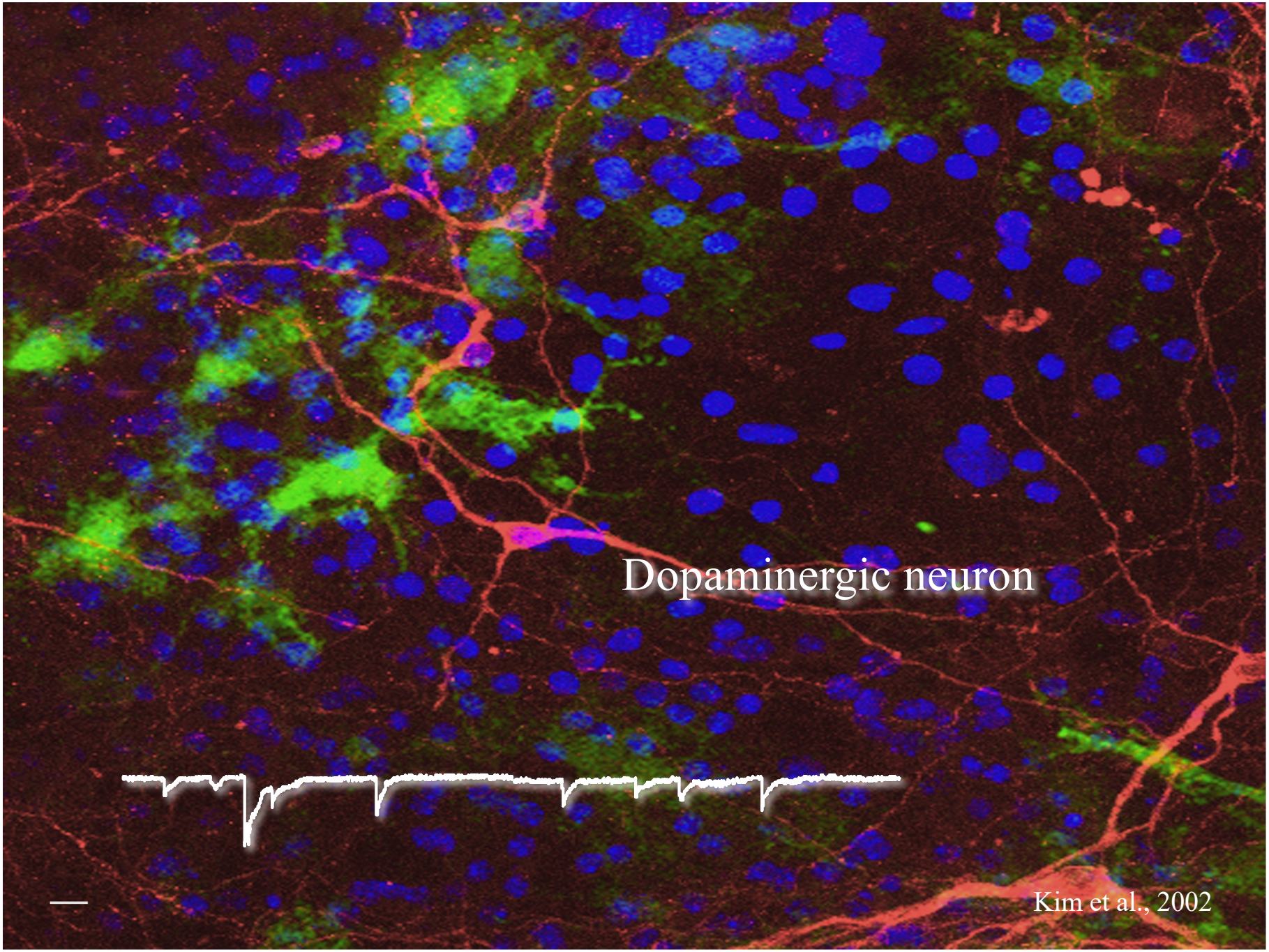
ES: in vivo differentiation



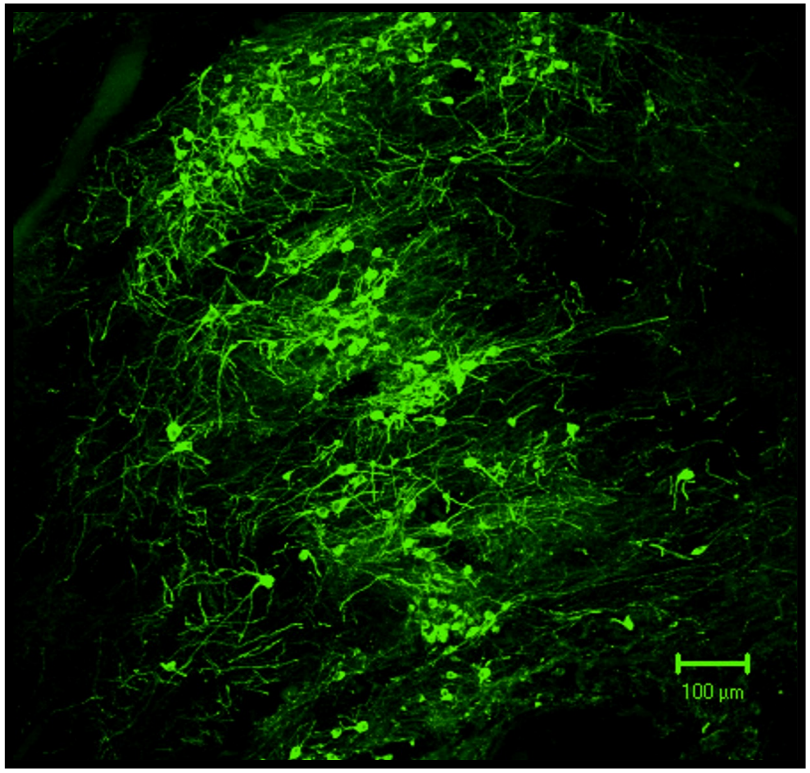
teratoma
(in mouse after transplantation)

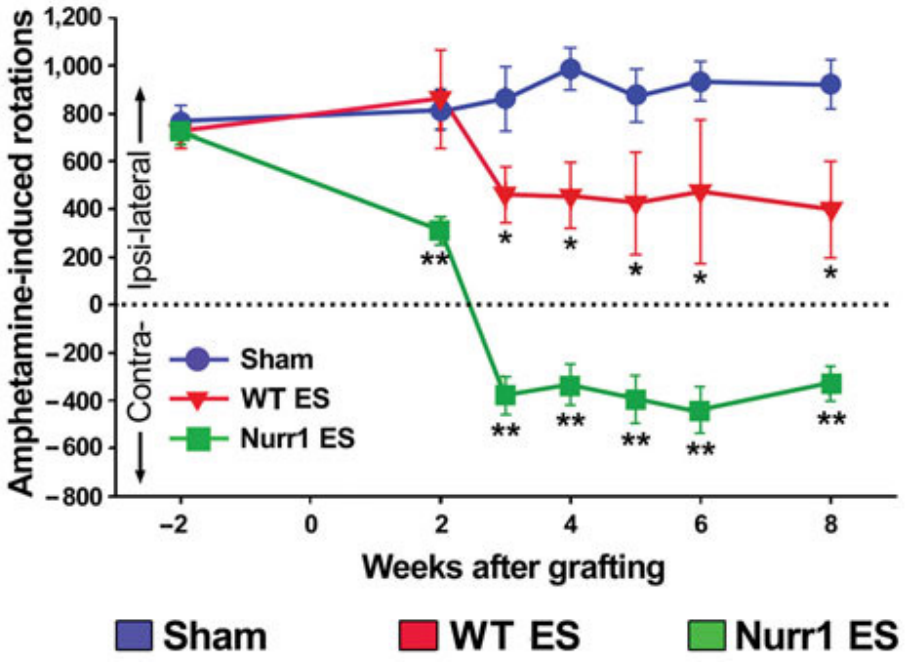
chimera
(implanted in the blastocyst)

Dopaminergic neurons from mouse ES differentiated in vitro



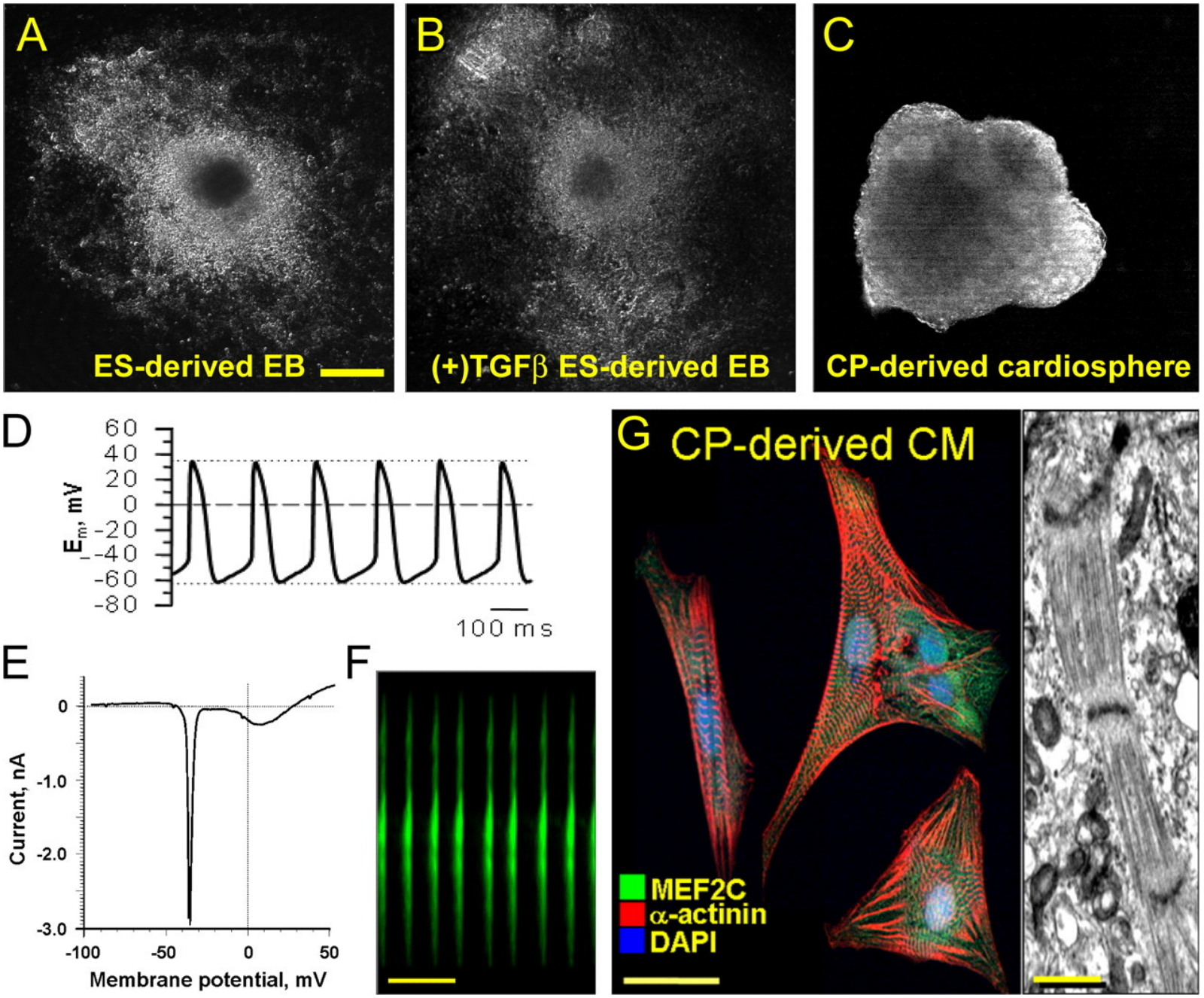
In vivo transplantation



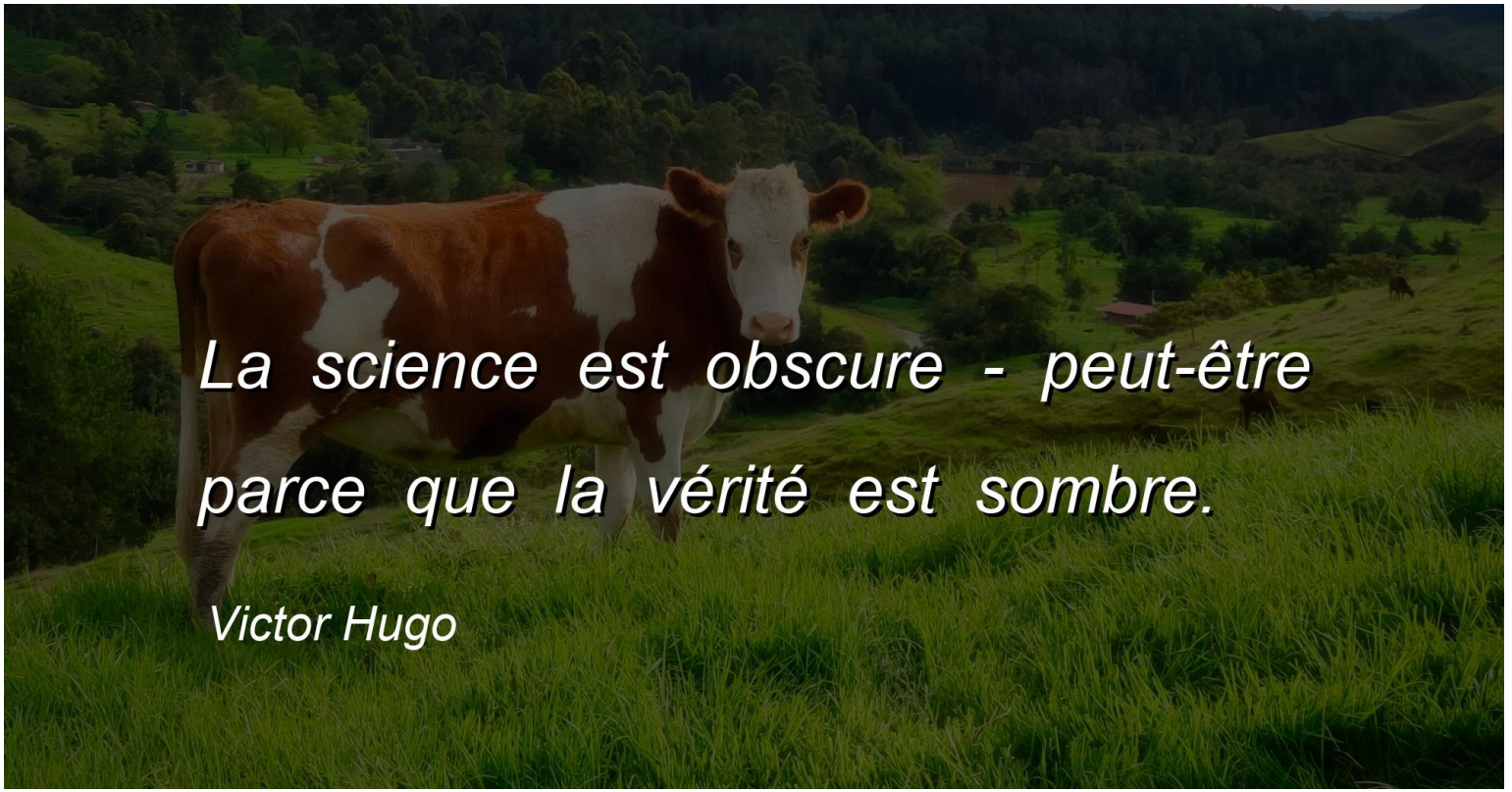


Kim et al., 2002 Nature ES murine

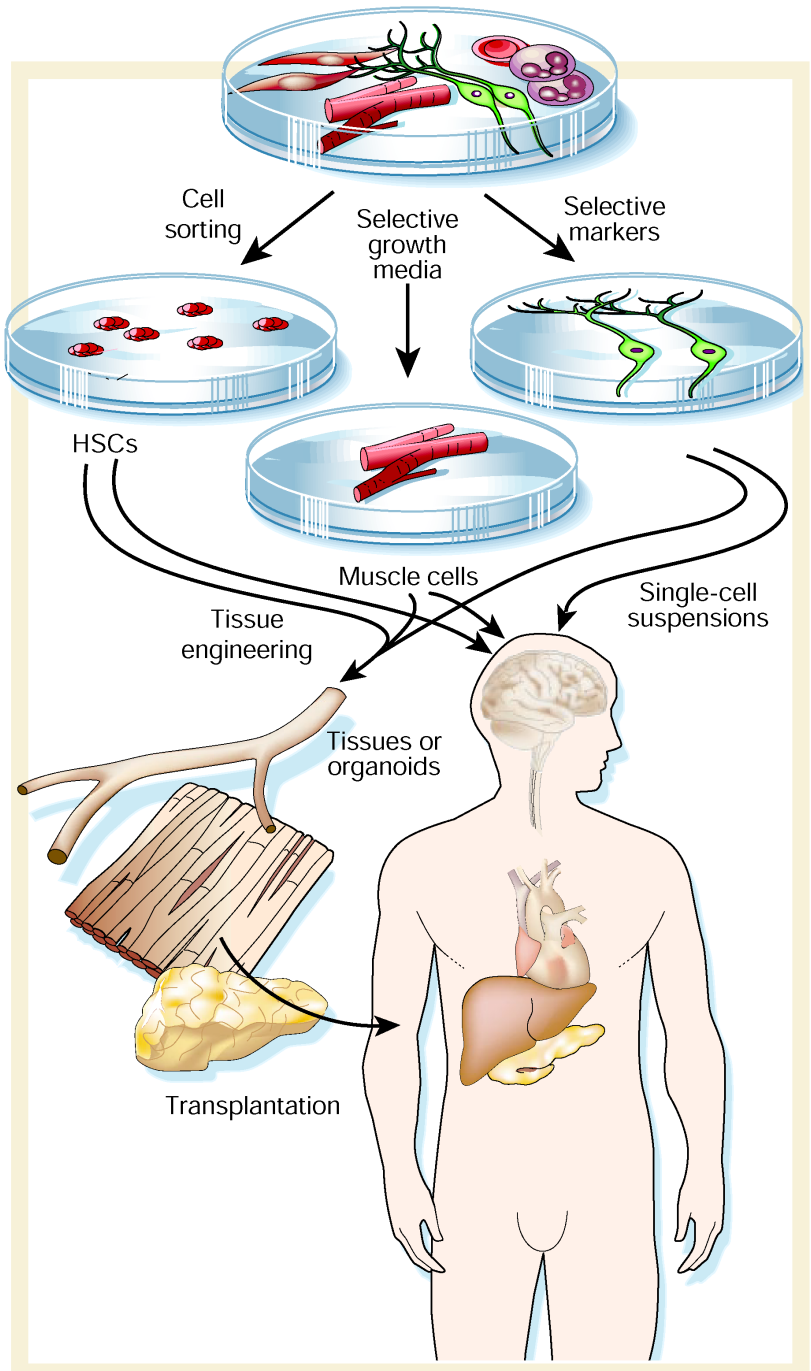
Cardiac cells from mouse ES



Behfar et al, 2007


La science est obscure - peut-être parce que la vérité est sombre.
Victor Hugo

ES: in therapy



ES: problems

A) Technical

manipulation

histocompatibility

……..

B) Bioethics

religion

laws

……..

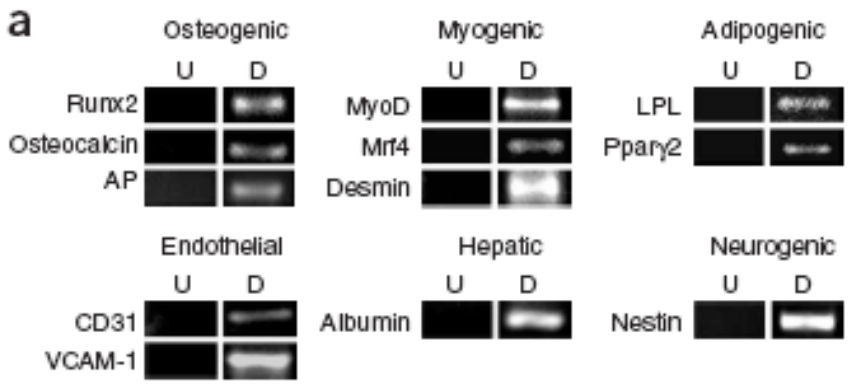
Stem cells from human embryos

(Europe: 100.000 spare frozen embryos from FIVET)

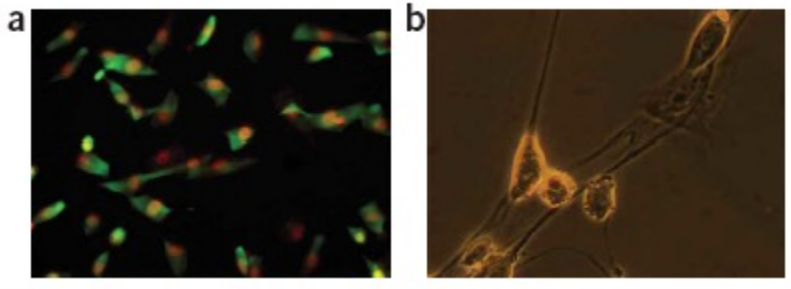
Germany	illegal
Great Britain	legal
Italy	illegal
Israel	legal
USA	Bush: illegal (cells derived before 9.08.2001)
	Obama: legal
	(Executive Order 13505 - Removing Barriers to Responsible Scientific Research Involving Human Stem Cells - March 9, 2009)

Napolitano Cattaneo 2013

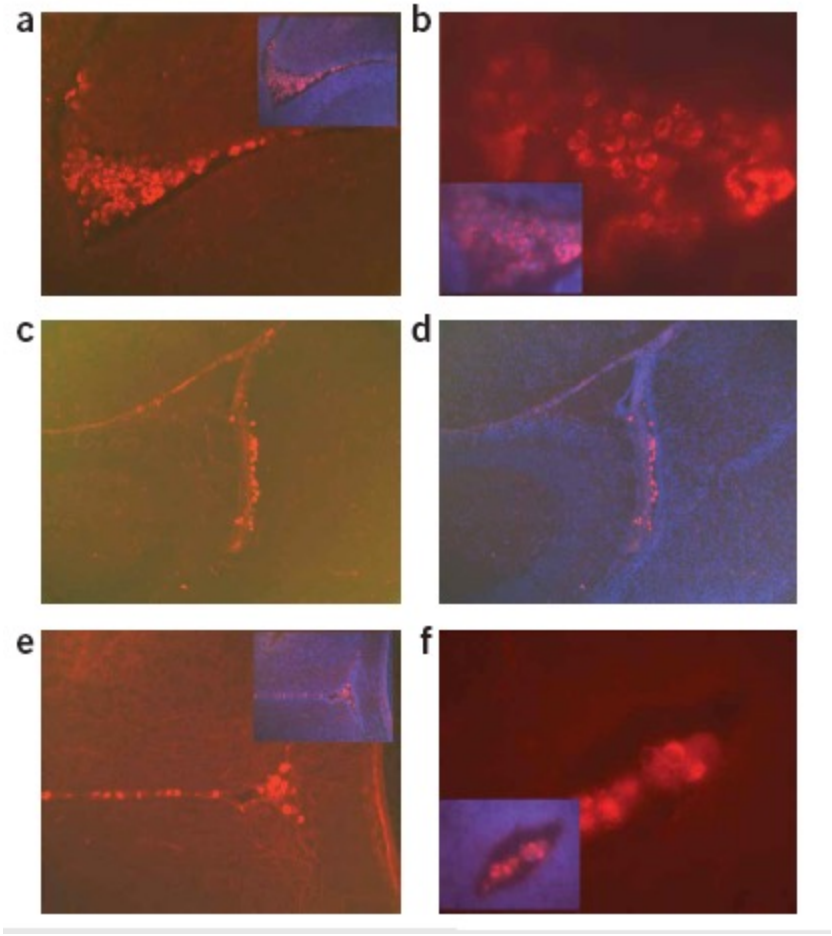
Amniotic fluid stem cells - *life in plastic is fantastic*



RT PCR for mRNA lineage specific



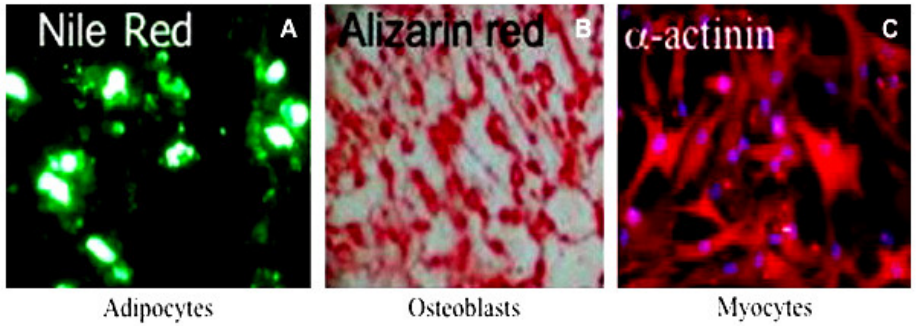
Neuron markers (nestin)



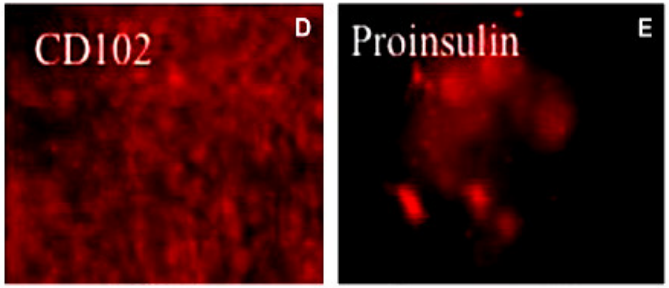
Mouse brain sections after implants of AFC: red hum mitoc

Coppi et al NBT 2007

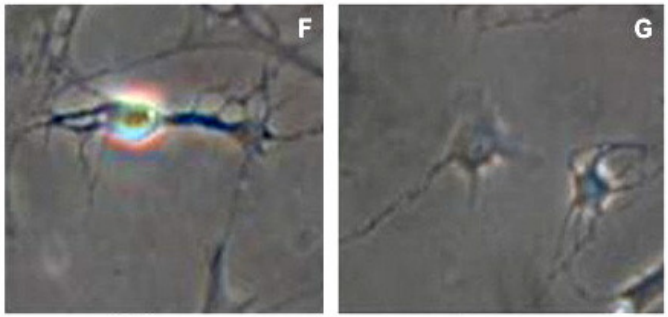
Umbilical cord blood cells (UCB)



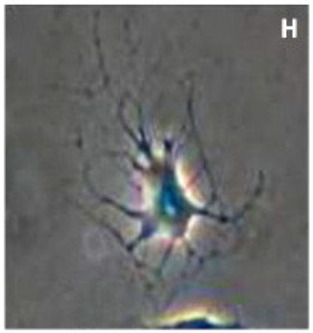
Adipocytes Osteoblasts Myocytes



Endothelium Hepato-pancreatic body



Neurons Astrocytes



- Hemopoietic cells

- Endothelial progenitors
- Mesenchymal progenitors
- Stem cells pluri/multipotent

Advantages:

•amount
•immunological “youth”
•banking

Van de Ven et al 2007

Post natal stem cells

Limited proliferation

Limited pluripotency

But……

How to isolate stem cells – localization of post natal stem cells

In regenerating tissues (blood and skin)

In low regenerating tissues (bone)

In non regenerating tissues (teeth, CNS)

How to isolate stem cells

- Antigen markers
- Efflux of the DNA binding dye Hoechst 33342

Best characterized adult stem cells: hematopoietic stem cells (HSC)- (Spangrude 1988)

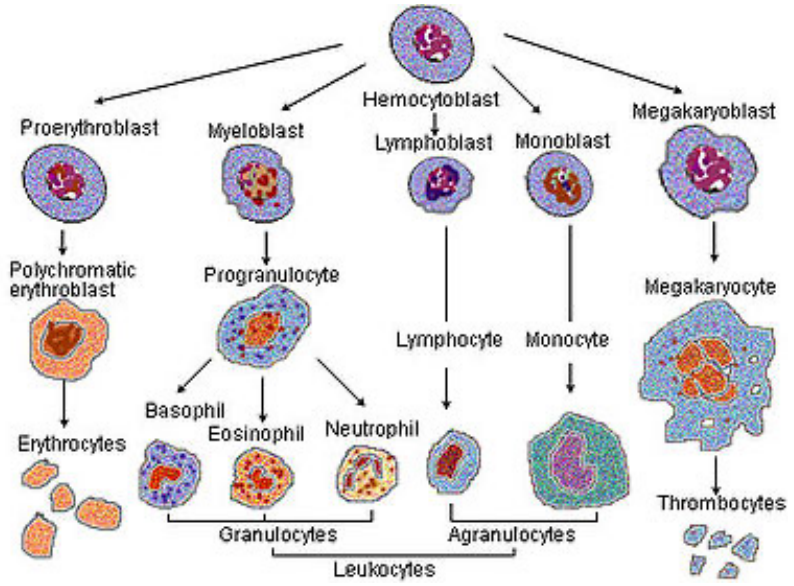
•Short term hematopoietic stem cells: 2 months

•Long term hematopoietic stem cells: greater than 6 months

•CD34+, enrichable on the basis of membrane markers 10000 -fold, 80% purity (and also marker negative selection)

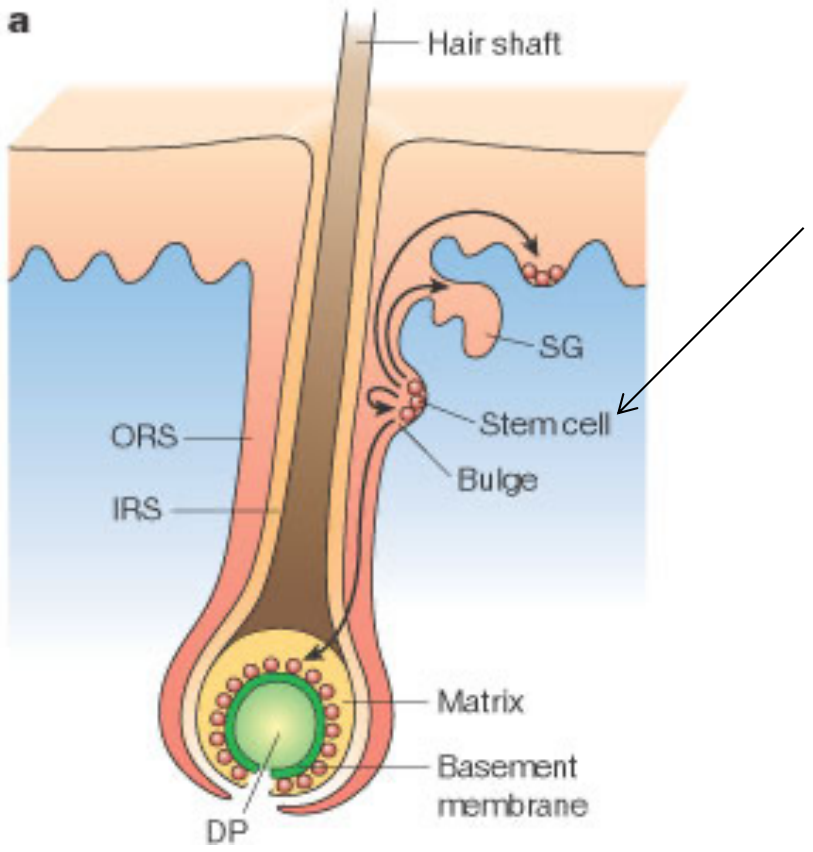
- **Mouse HSC :** $CD34^{lo/-}$, $SCA\text{-}1^{+}$, $Thy1.1^{+/lo}$, $CD38^{+}$, $C\text{-}kit^{+}$, lin^{-}
- **Human HSC :** $CD34^{+}$, $CD59^{+}$, $Thy1/CD90^{+}$, $CD38^{lo/-}$, $C\text{-}kit/CD117^{+}$, lin^{-}

•Can reconstitute **blood**

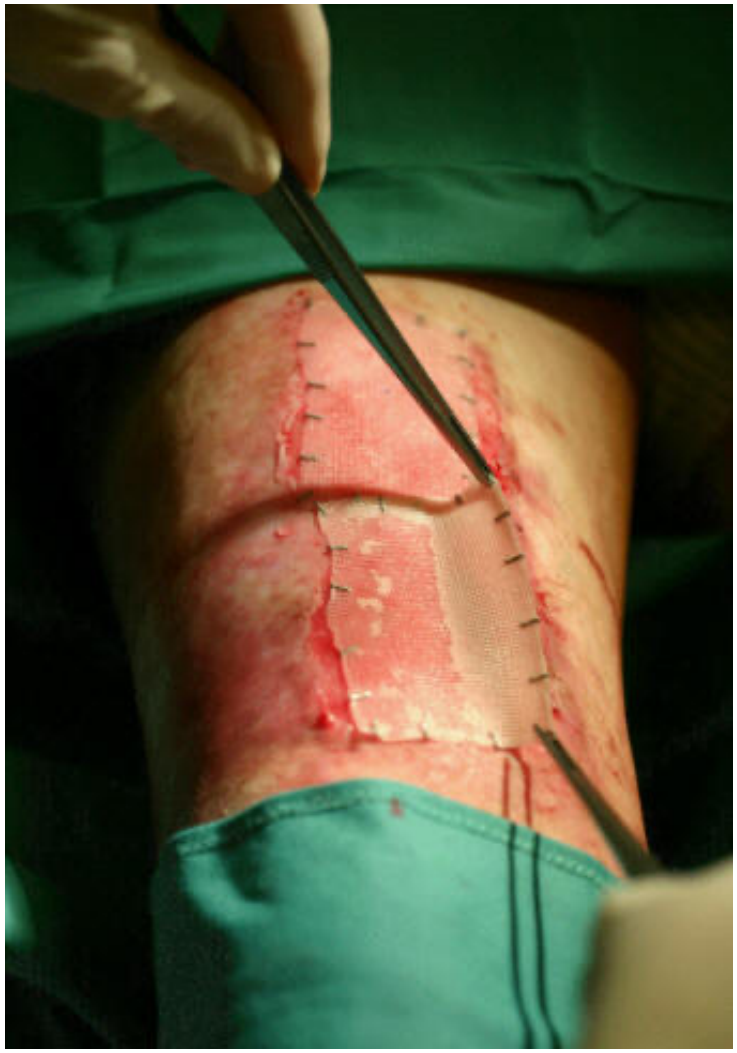


Stem cells for skin

- Hair follicles keratinocytes
- expressing keratin K5 and K14



Stem cells for skin



Michele De Luca

Write a summary of 20 lines for a general public

Molecular Therapy
Original Article

Allele-specific CRISPR-Cas9 editing of dominant epidermolysis bullosa simplex in human epidermal stem cells

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[1]Centre for Regenerative Medicine "Stefano Ferrari", Department of Life Sciences, University of Modena and Reggio Emilia, 41125 Modena, Italy; [2]Holostem Terapie Avanzate, s.r.l, 41125 Modena, Italy; [3]Department of Life Sciences, University of Modena and Reggio Emilia, 41125 Modena, Italy; [4]Department of Biochemical Sciences 'A. Rossi Fanelli', Sapienza Università di Roma, 00185 Rome, Italy; [5]Genomic Units, IRCCS Humanitas Research Hospital, 20089 Rozzano, Milan, Italy; [6]Department of Medical and Surgical Sciences, University of Modena and Reggio Emilia, 41124 Modena, Italy; [7]Regenerative and Oncological Dermatological Surgery Unit, Modena University Hospital, 41124 Modena, Italy

Epidermolysis bullosa simplex (EBS) is a rare skin disease inherited mostly in an autosomal dominant manner. Patients display a skin fragility that leads to blisters and erosions caused by minor mechanical trauma. EBS phenotypic and genotypic variants are caused by genetic defects in intracellular proteins whose function is to provide the attachment of basal keratinocytes to the basement membrane zone and most EBS cases display mutations in keratin 5 (*KRT5*) and keratin 14 (*KRT14*) genes. Besides palliative treatments, there is still no long-lasting effective cure to correct the mutant gene and abolish the dominant negative effect of the pathogenic protein over its wild-type counterpart. Here, we propose a molecular strategy for EBS01 patient's keratinocytes carrying a monoallelic c.475/495del21 mutation in *KRT14* exon 1. Through the CRISPR-Cas9 system, we perform a specific cleavage only on the mutant allele and restore a normal cellular phenotype and a correct intermediate filament network, without affecting the epidermal stem cell, referred to as holoclones, which play a crucial role in epidermal regeneration.

INTRODUCTION

Inherited epidermolysis bullosa (EB) is a heterogeneous group of rare, autosomal genetic disorders caused by molecular defects within genes encoding structural proteins forming the epidermal-dermal junction. EB is characterized by recurrent blistering and erosions of the skin (and other stratified epithelia) that arise, spontaneously or upon minimal mechanical stress, within the epidermis in EB simplex (EBS), the lamina lucida in junctional EB (JEB) and beneath the lamina densa in dystrophic EB (DEB). EBS is the most common EB form, with a prevalence of 1 in 30,000 to 1 in 50,000.[1,2] Its clinical manifestations are usually less severe than those of JEB and DEB, which can be devastating and even early lethal. However, some EBS forms are marked by a severe phenotype and several clinical variants have been identified based on the mutated gene, site of blister formation, anatomical distribution, and mode of inheritance.[3–5]

JEB and DEB are mostly recessively inherited, while the vast majority of EBS are inherited in a dominant manner. In fact, approximately 75% patients suffering from EBS harbors dominant mutations in *KRT5* and *KRT14*, the genes encoding keratin 5 (K5) and keratin 14 (K14), respectively. K5/K14 pairs form the basal keratinocyte intermediate filaments, which are part of the hemidesmosomal protein complex tethering the epidermal basal layer to the basement membrane and the underlying dermis. Mutant keratins exert a dominant negative effect on the functional keratins encoded by the normal allele, hence perturbing the basal keratinocyte intermediate filament network and leading to intraepidermal blister formation. Thus, while JEB and DEB can be tackled by the addition of a corrected copy of the mutated gene in the genome of epidermal stem cells,[6–14] a potentially successful combined *ex vivo* cell and gene therapy of EBS strictly requires editing of the mutated allele.

Here, we outlined an allele-specific CRISPR-Cas9-based gene-editing approach that is able to disrupt specifically the *KRT14* mutant gene and fully restore functional intermediate filaments in epidermal stem cells cultivated from an EBS patient carrying a *de novo* monoallelic c.475/495del21 dominant mutation in exon 1 of *KRT14*.

This approach takes advantage of a tailored CRISPR-Cas9 system to induce double-strand breaks (DSBs) specifically on the mutant allele, leading to non-homologous end-joining (NHEJ) repairing process. These rearrangements are likely to generate frameshift mutations resulting in both pathogenic allele expression abolishment and phenotypic and mechanical stress resilience restoration. Besides the

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