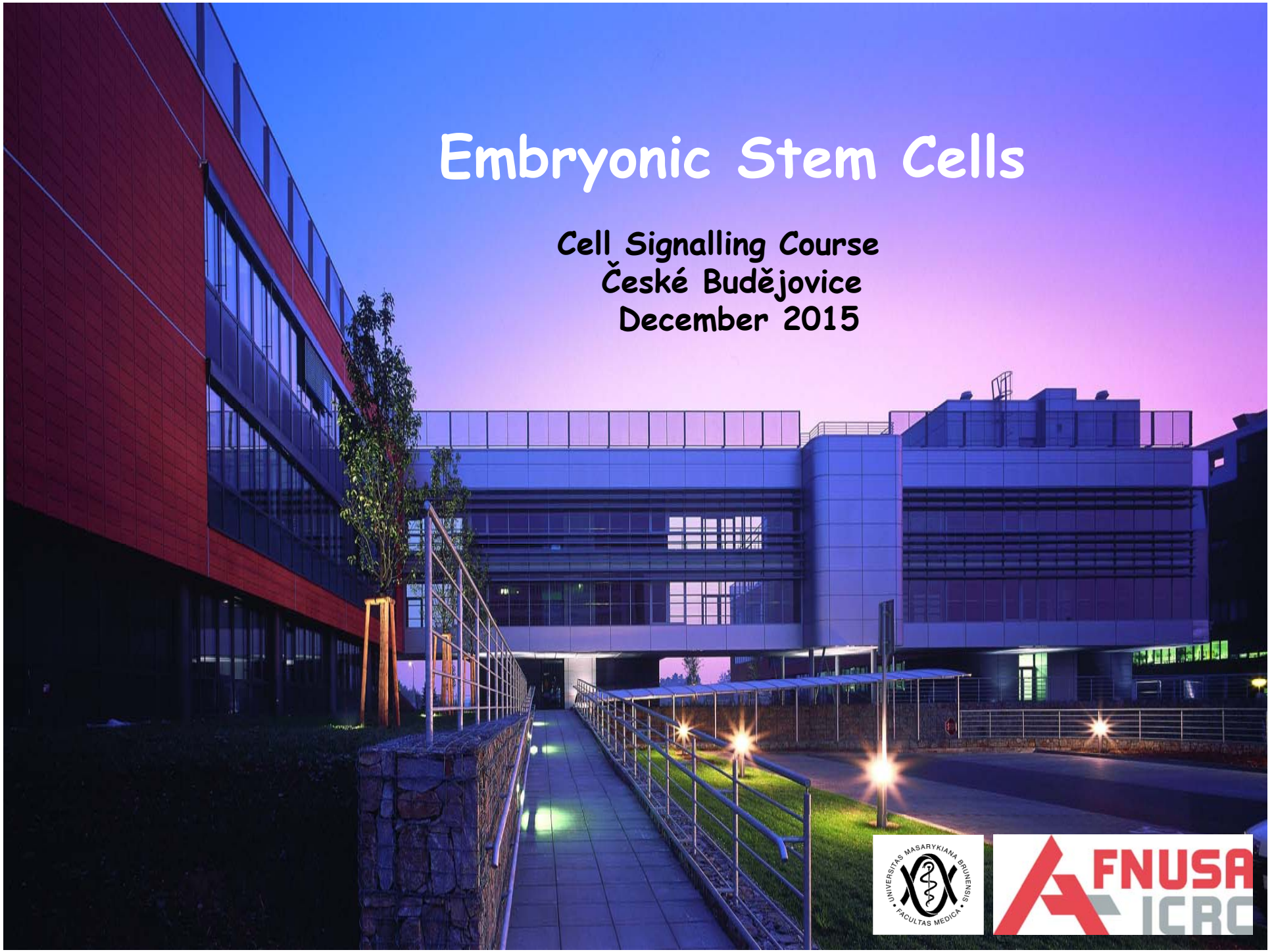
Embryonic Stem Cells

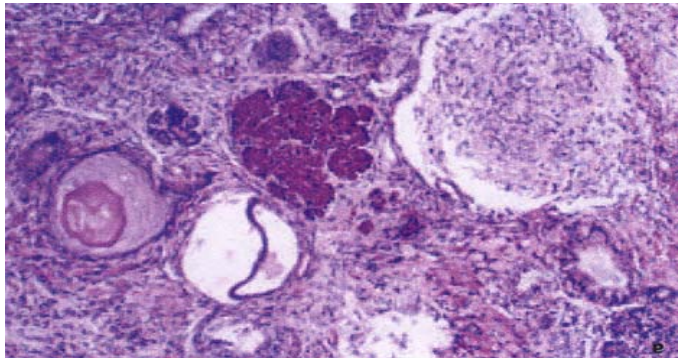
Cell Signalling Course
České Budějovice
December 2015



Pluripotent /multipotent stem cells

(Embryonic, Adult, Induced,…?)

Where can we find the origins of stem cell research?



Tumours were at the beginning

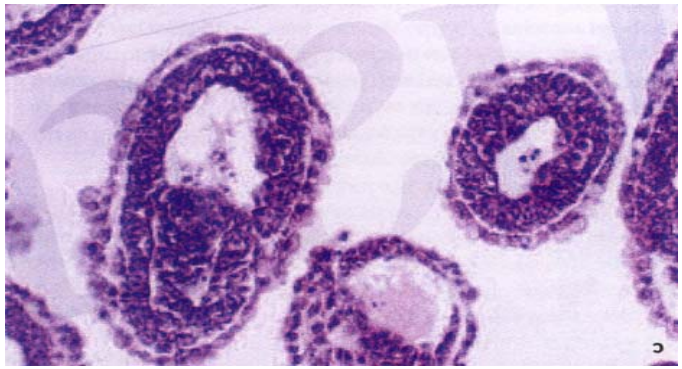
(teratomas/teratocarcinomas)

1954 – mouse strain 129, spontaneous development of testicular teratocarcinomas (Stevens & Little)

Key finding

1964- teratocarcinomas contain individual cells that have the capacity to differentiate into many different cell types (Kleinsmith & Pierce)

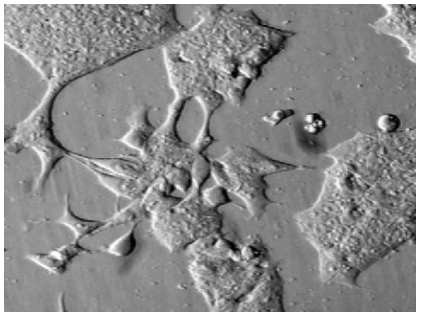
PLURIPOTENCY



Gail Martin

Martin Evans

Cells of teratocarcinomas bring another important finding.



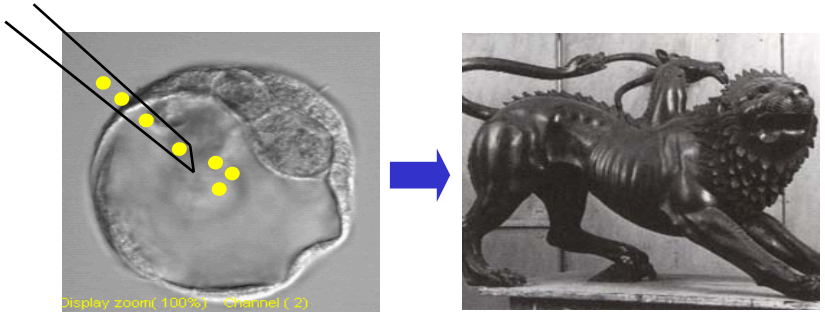
Growth without a loss of pluripotency

1974 – cells of teratocarcinomas maintain their pluripotency when propagated *in vitro* (Gail Martin & Martin Evans)

SELF-RENEWAL

Another example of pluripotency

1974 – chimaeras are produced upon injection of cells of teratocarcinomas into blastocyst-stage embryo (Martin & Evans)

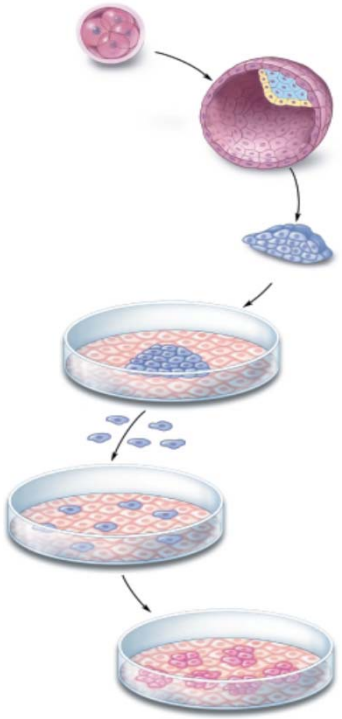


1981

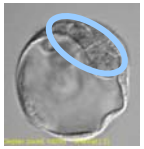
Lines of pluripotent cells were established for the first time from mouse embryo – Embryonic Stem Cells

(Martin & Evans)

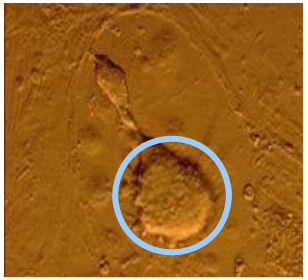
Embryonic Stem Cells (ESC) – step from cancerous pluripotent cells of teratocarcinomas to „normal" pluripotent cells



Early embryo at blastocyst stage

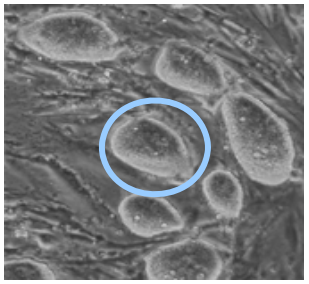


Isolated embryoblast (ICM - Inner Cell Mass)



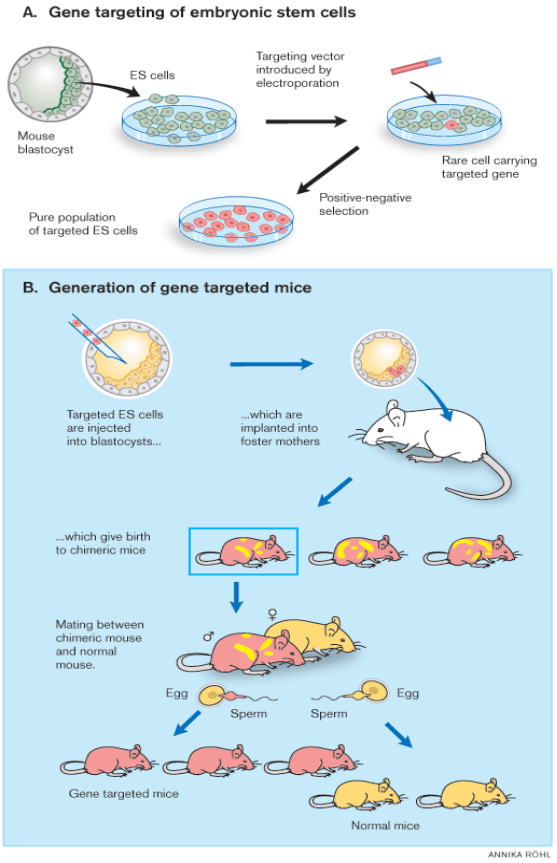
Isolated embryoblast after placing to *in vitro* conditions (+ feeder cells + LIF)

Propagation in culture by enzymatic disaggregation (repeated passaging)



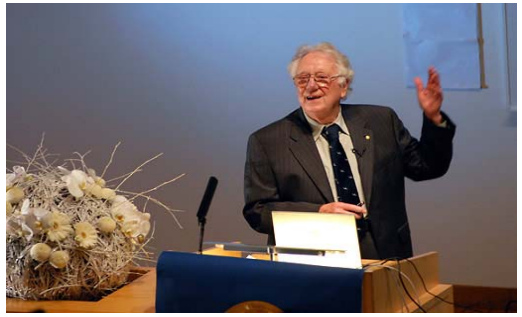
The Nobel Prize in Physiology and Medicine 2007

Development of techniques to make knockout mice using **ES cells** that offered an opportunity to generate live animals with a desired mutation in every cells!



Sir Martin Evans

Mario R. Capecchi

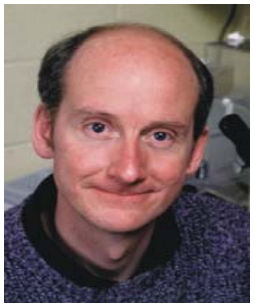


Oliver Smithies

over 35 000 papers

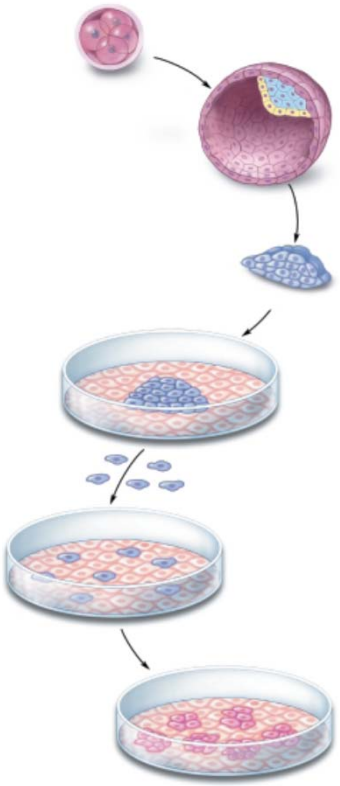
The history of embryonic stem (ES) cells.

• The establishment of mouse embryonal carcinoma (EC) cells	Martin, 1975
• The establishment of mouse embryonic stem (ES) cells	Martin, 1981
• The isolation of totipotent (?) bovine embryonic stem cells	Sins, 1993
• The culture of pig ICM-derived cells	Strojek, 1990 Wheeler, 1994
• The culture of sheep ICM-derived cells	Galli, 1991 Moor, 1992
• The culture of rabbit ICM-derived cells	Giles, 1993 Dvorak, 1997
• The isolation of primate embryonic stem cells	Thomson, 1995
• The isolation of human embryonic stem cells	Thomson, 1998 Reubinoff, 1998



Human Embryonic Stem (hES) Cells.

(Thompson et al, 1998)



Early embryo at blastocyst stage

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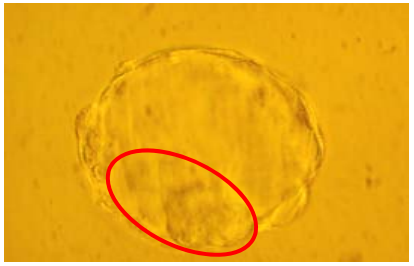
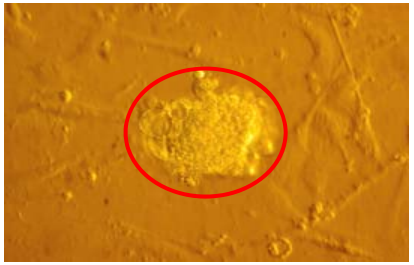
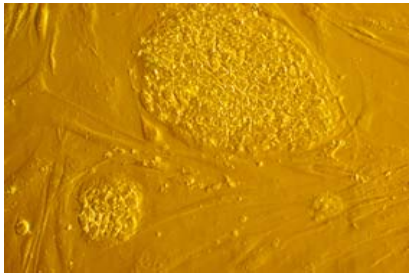
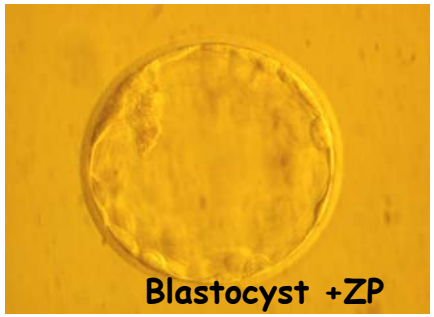






Technology to obtain human ES cell line.

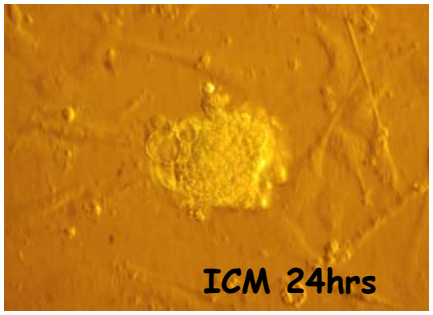


Early embryo – blastocyst
(human 4-5 days, mouse 3.5 days)

- **donated for research purposes – written consent**
- **no monetary compensation**

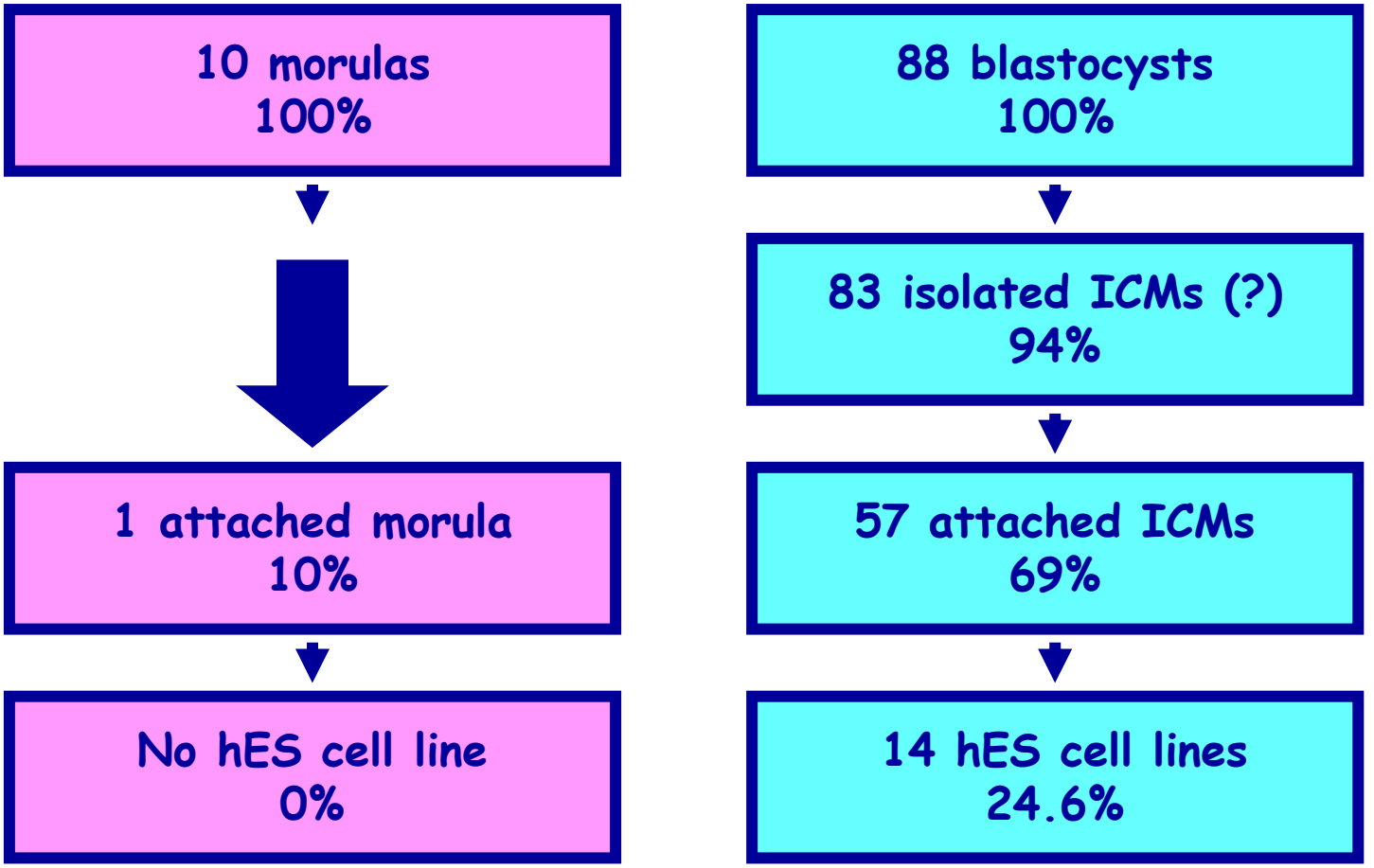
Blastocyst = trophectoderm + **ICM (embryoblast)**

- **Removal of zona pellucida** (pronase treatment)
- **Isolation of ICM by immunosurgery**
- **Placing ICM onto feeder layer of MEFs** (CF-1)

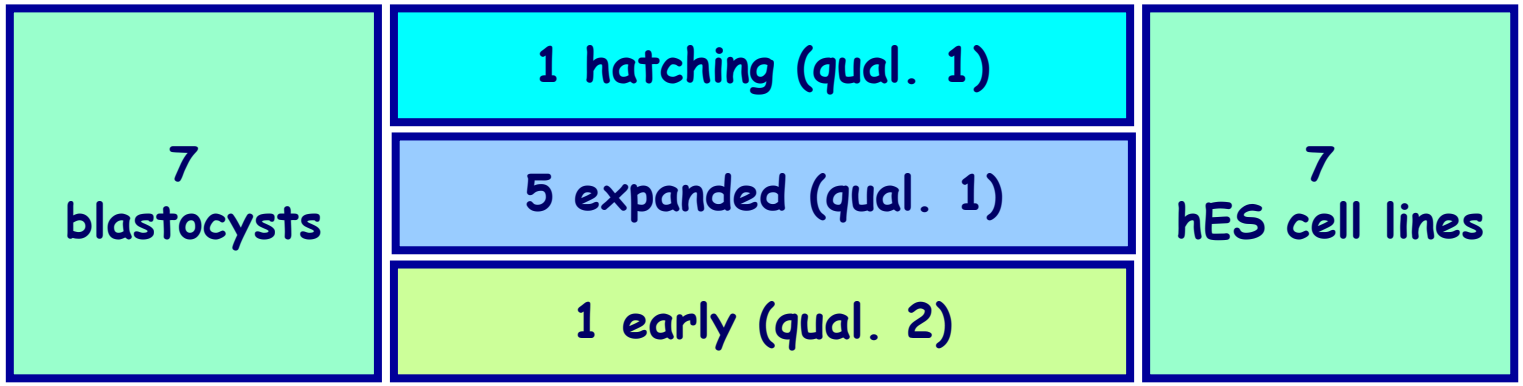


- **Culture in appropriate media**
  (DMEM/F-12 with KO-SR and FGF-2)
- **Hoping for a attachment of ICM**

Derivation of hESC is not a 100% success process



Quality of embryo matters



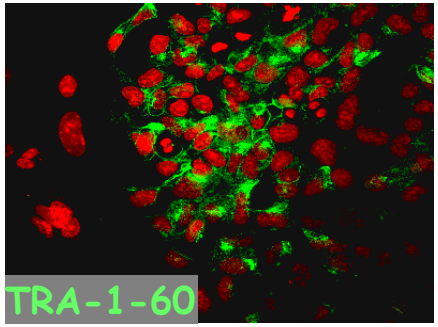
Behavior of embryoblast in culture varies

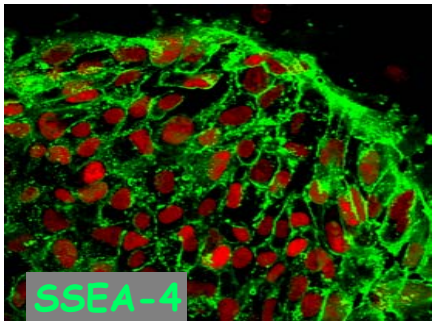
hESC line	the first outgrowth at day
CCTL1	8
CCTL2	9
CCTL3	4
CCTL4	5
CCTL5	8
CCTL6	3
CCTL7	8

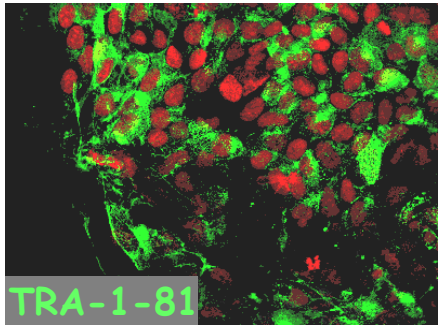
…as well as many other parameters

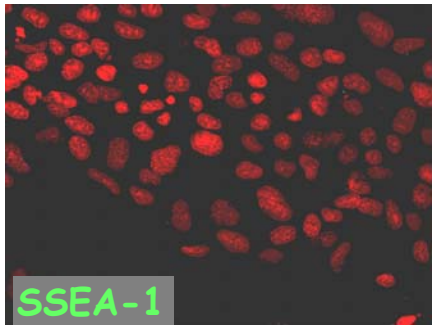
What can we use to evaluate embryonic stem cells?

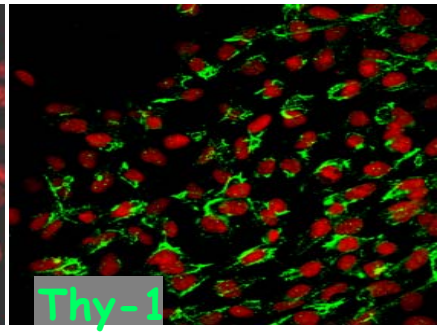
Molecular markers of pluripotency

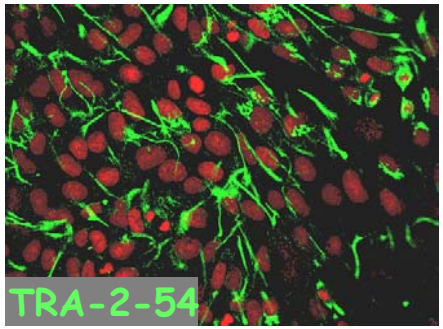


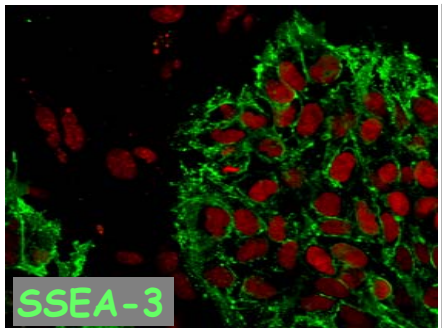




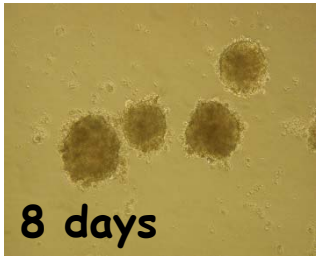


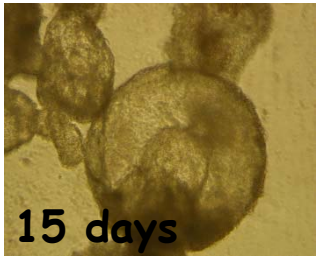


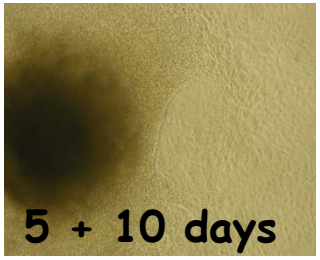




Capability to differentiate







CCTL12

Wide variety of stem cells.

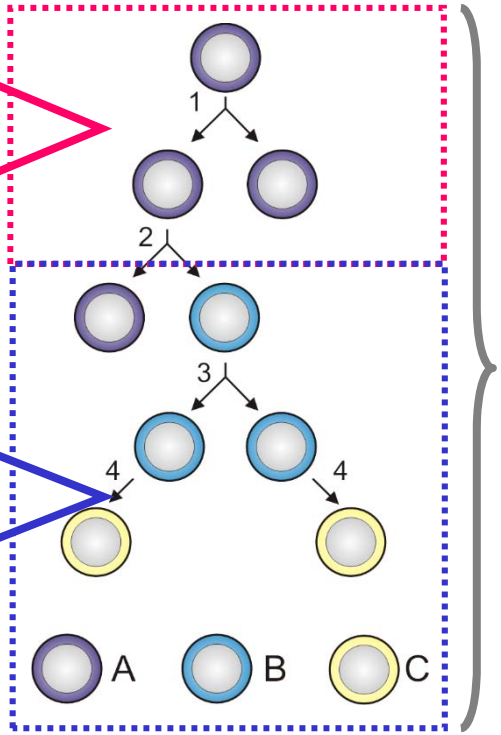
Stem cells generate and regenerate our body

Capability to produce identical copies of itself

Self-renewal

Capability to differentite into specialized cell types

Pluripotency



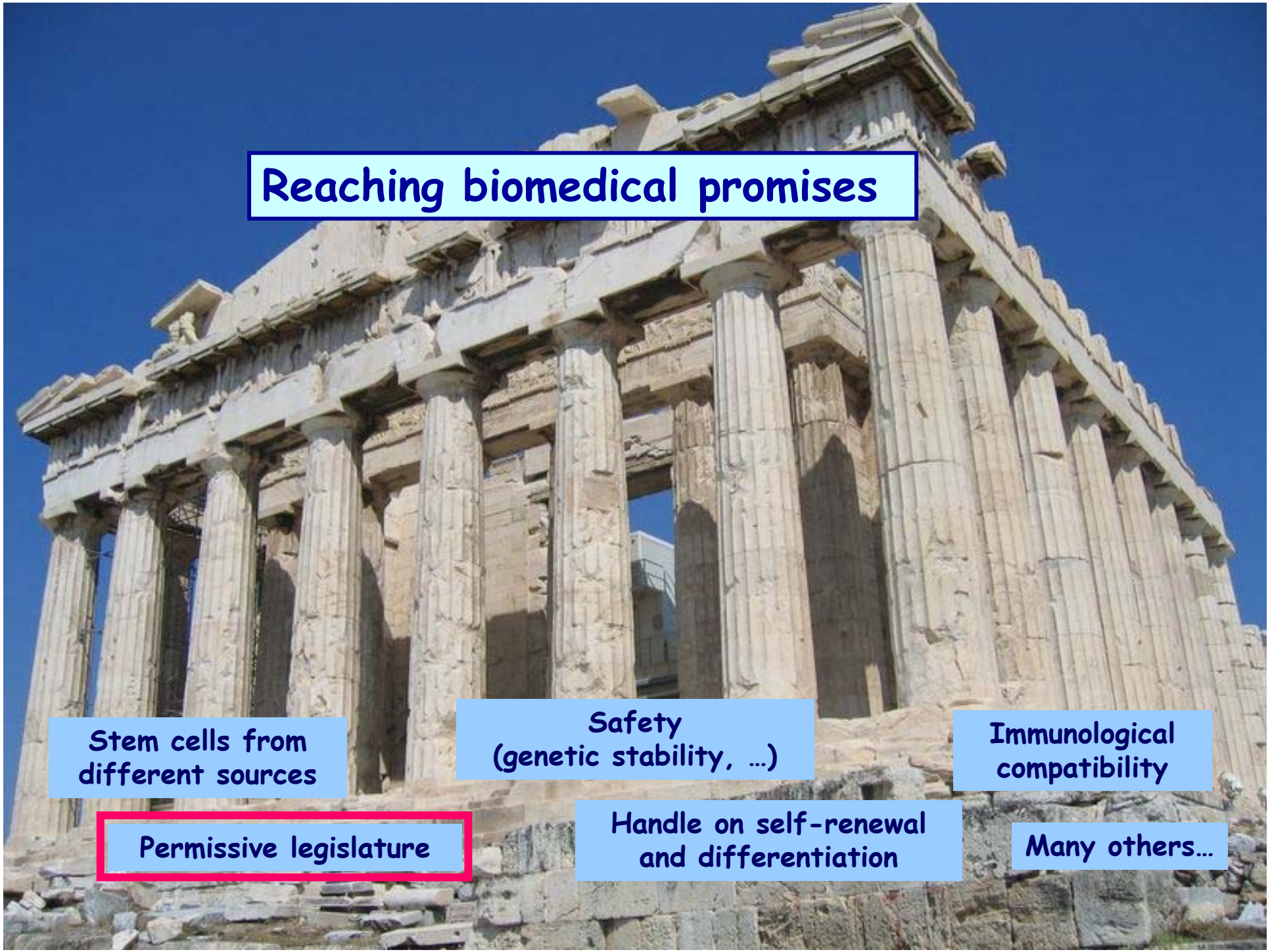
- Embryonic stem cells
- Adult stem cells
  - Fetal
  - Organ
  - Tissue
- Induced pluripotent stem cells

- Cancer stem cells

Different properties

Pluripotent /multipotent stem cells

(Embryonic, Adult, Induced,…?)


Reaching biomedical promises
Stem cells from different sources
Safety (genetic stability, …)
Immunological compatibility
Permissive legislature
Handle on self-renewal and differentiation
Many others…

What is the legal status of experimenting with human ES cells in the Czech Republic?

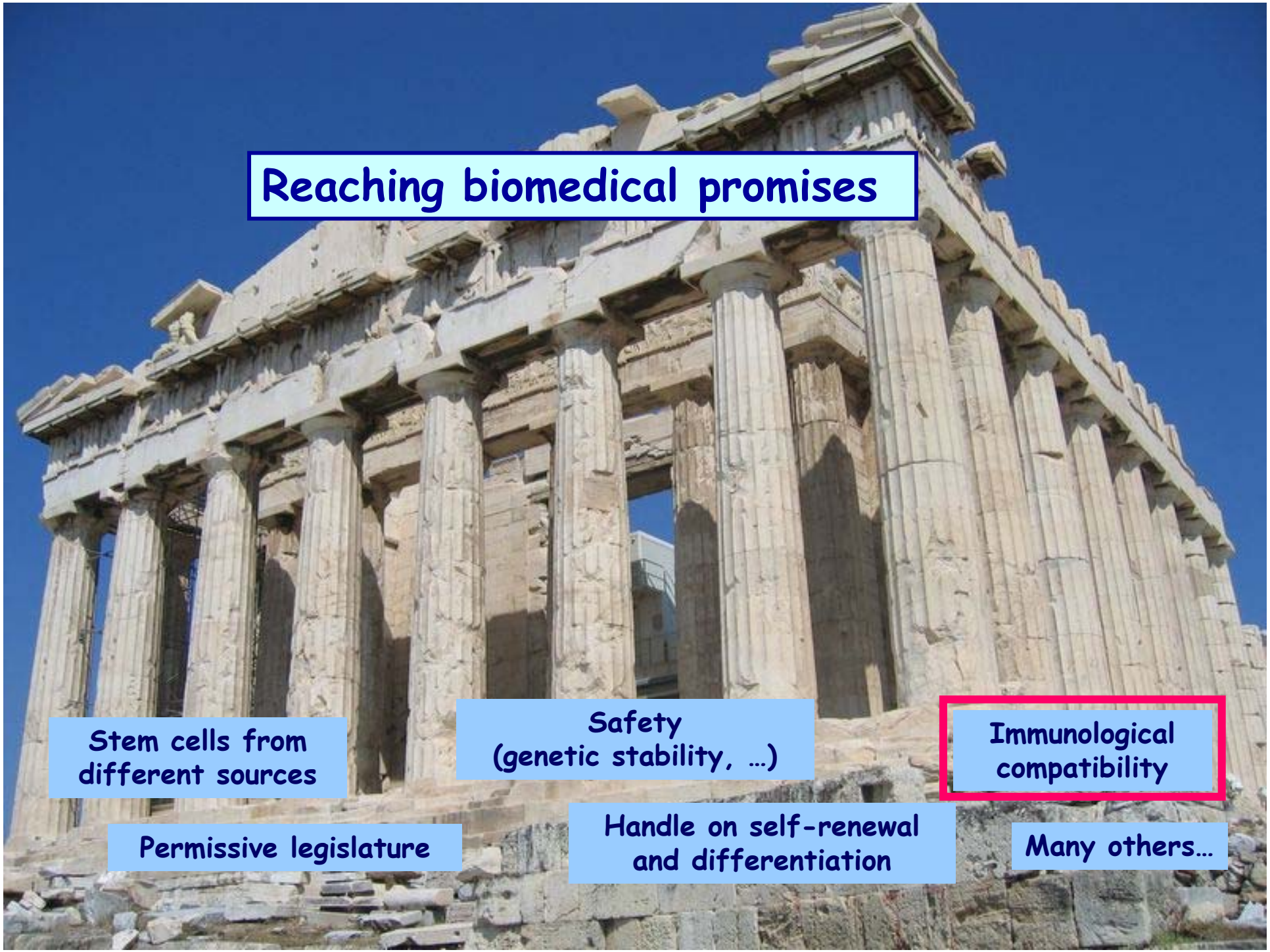
Permissive

Act on research on human embryonic stem cells and related activities and on amendment to some related acts

Passed by Parliament of the Czech Republic on April 26, 2006

In effect since June 1, 2006 as Act no. 227/2006 Coll.

Permisssion for work with human embryonic stem cells
Czech registry of human embryonic stem cells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How many lines of human ES cells do we really need ?

Two aspects

Experimenting on hESC

- „Normal" lines
- Genetically abnormal lines

Potential use of HESC in regenerative medicine

- Manipulation without xenogenic substances
- Imunological compatibility between ESC and patient (about 1500 aleles in 12 HLA loci)

USA

Federal funds – only lines that were derived before August 9, 2001, 9:00 a.m.

78 lines complied with this criteria - only 22 lines were in fact available

Are all the lines af human embryonic stem cells „the same“ ?

NO

Differences

- growth properties *in vitro*
- differentiation properties

Osafune et al. Marked differences in differentiation propensity among hESC lines. Nature Biotech, 2008

Sources of differences

The way of manipulation with hES cells (derivation, propagation,…)

Biology of indiviual human embryos !

International Stem Cell Initiative

(International Stem Cell Forum – Prof. Peter Andrews)

Centre for Stem Cell Biology & UK Stem Cell Bank

collaboration and funding support for stem cell research (**Table 1**). The International Stem Cell Initiative grew out of a meeting held under the auspices of the Forum in London in May 2003. The meeting brought together experts in hES cell research from around the world to plan an international collaborative effort to establish a set of standards for the characterization of hES cell lines.

The group decided to begin with what was envisioned to be a relatively simple project, namely, to collect as many hES cell lines as possible and carry out a basic set of characterization studies on them under defined conditions. The exercise, which is supported by funding from the Forum members, is being conducted with the cooperation of the UK Stem Cell Bank as a central hub for collection and distribution of materials. Forum members were invited to nominate laboratories to submit their hES cell lines to the Initiative. Prospective participating laboratories were asked to certify that their hES cell lines had been derived following generally accepted ethical guidelines and to agree that all information generated by the Initiative would be placed in the public domain. Seventeen laboratories from 11 Forum member countries agreed to participate and are contributing a total of 75 hES cell lines to the study (**Fig. 1**). These laboratories are carrying out surface-antigen expression analyses on their own cells and preparing nucleic acids and other samples for study by several other central reference laboratories.

Characterization studies

The studies include flow cytometric analysis of the expression of 17 surface antigens, quantitative RT-PCR analysis of the transcript levels of ~100 genes characteristic of pluripotent stem cells and their early differentiated derivatives, and an examination of how the expression pattern of these ~100 genes changes in response to a simple differentiation protocol involving embryoid body formation. The antigens chosen are those commonly used by many groups to define hES cells. They include markers such as SSEA3, SSEA4, *TRA-1-81* and the antigens defined by antibodies TRA-1-60 and GCTM2, all of which have been previously reported to be characteristically expressed by undifferentiated hES cells. To ensure standardization, agreement was reached with the owners of all the key hybridomas that define these marker antigens to deposit them in an archive at the National Institute of Biological Standards and Control in the UK, the home of the UK Stem Cell Bank and a WHO Reference Laboratory.

The gene expression studies are focused on molecules that are widely reported to be good markers of human pluripotent stem cells, including some whose functions are likely essential to maintenance of pluripotentiality, such as *POU5F1* (also known as *OCT4*), *NANOG*, *SOX2*, *ZFP42* (also known as *REX1*), *UTF1*, *GDF3*, *FOXD3*, *TERT*, *FGF4*, and others, such as *LIFR* and *LRPPRC* (also known as *GP130*), whose role in maintaining pluripotentiality is more controversial. Also included in the analysis are genes whose expression marks particular differentiation lineages, for example, *T* (also known as *BRACHYURY*, mesoderm), *MYF5* and *MYOD1* (muscle markers), *GATA4* (endoderm), *TAT* (hepatocytes), and *INS* (pancreatic beta cells).

Additional studies are aimed at assessment of the epigenetic status of the cell lines (expression of imprinted genes), examination of spatial patterns of marker expression in growing colonies by immunostaining *in situ*, and histological evaluation of teratomas formed by the cell lines. In addition, each line will be subjected to DNA fingerprinting, to provide definitive markers for identifying each line in future studies, and to microbiological analysis that will include a screen for possible endogenous retrovirus expression. Karyotyping will not be performed, but participants will be asked to provide karyotype data for each of their lines. Likewise, although the Initiative will not examine xenograft tumor production, participating laboratories have been invited to submit histological slides of any xenografts that they have produced from their lines for review by a histopathologist with expertise in this area.

The first examination of the preliminary dataset will take place at a two-day meeting of the Initiative participants at the Jackson Laboratory, in Bar Harbor, Maine, in August 2005. The entire analysis should be completed by the end of 2005. All the data will be placed in the public domain and will be available from the Forum website.

Goals of the Initiative

What are the expected outcomes of this first phase of the Initiative? Most researchers anticipate that expression of canonical cell-surface markers and pluripotency genes will be fairly consistent across the panel of cell lines, but in fact an exercise on this scale may turn up outliers with highly informative properties.

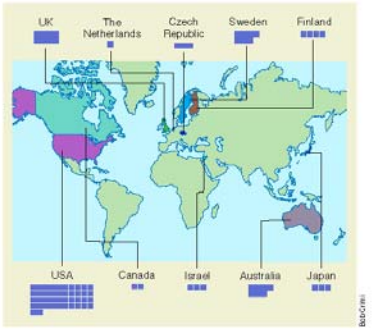


Figure 1 Countries of origin of hES cell lines in the Initiative. Blocks indicate number of hES cell lines contributed by each country.

Table 1 Members of the International Stem Cell Forum

Countries	
Australia[a]	Japan[a]
Canada[a]	Netherlands[a]
Czech Republic[a]	Singapore[a]
Denmark	Sweden[a]
France	Switzerland
Germany	UK[a]
Finland[a]	USA[a]
Israel[a]	
International member	
Juvenile Diabetes Research Foundation	

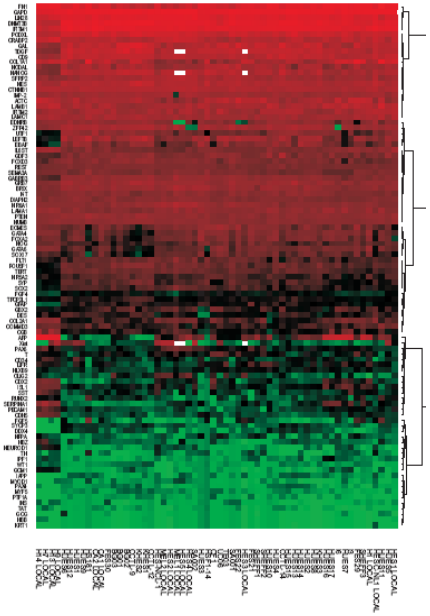
[a]Included in the Initiative are 75 hES cell lines derived in 17 laboratories from these Forum members.

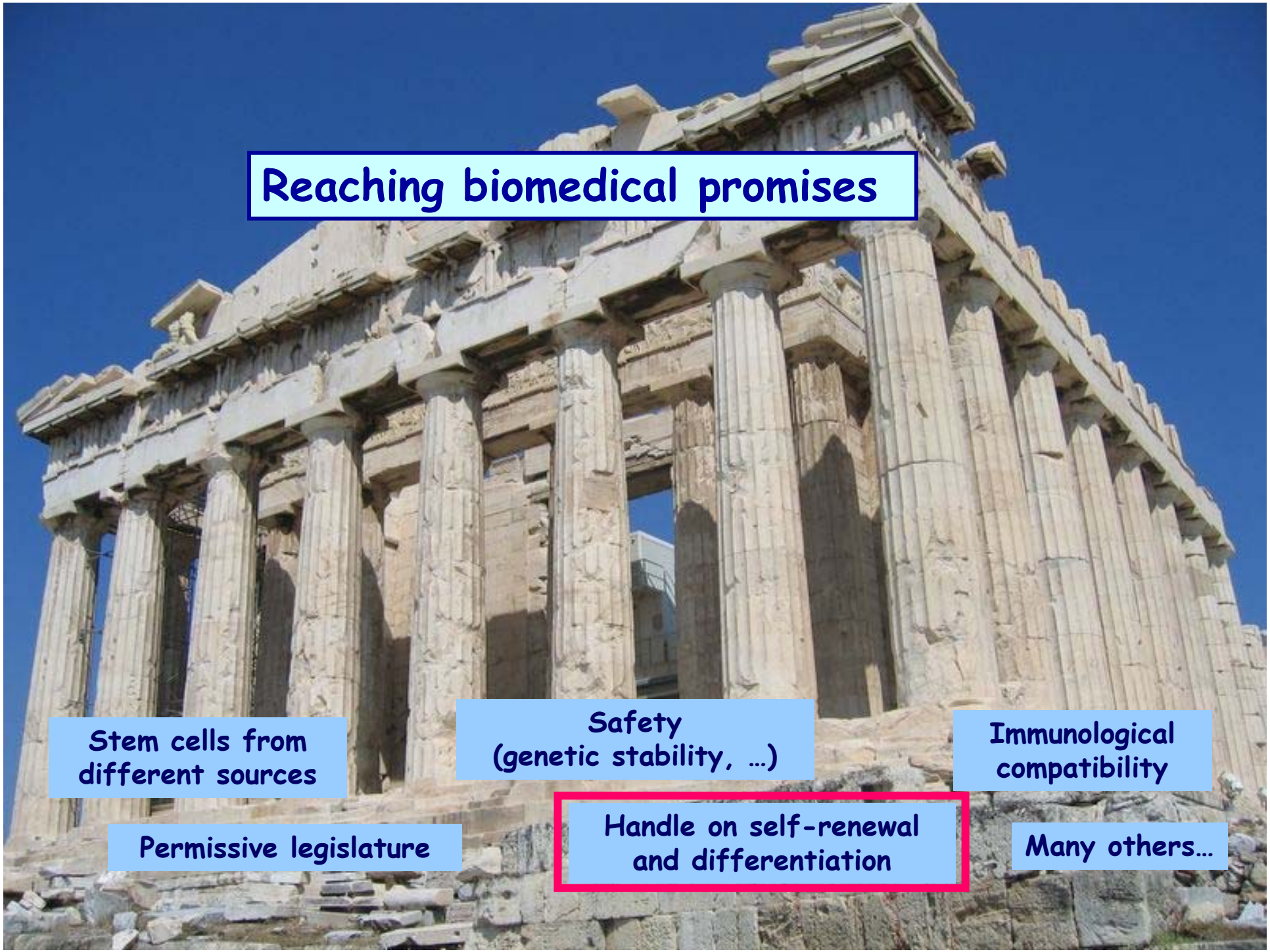
1. Antigen expression – FACS (nondif + dif)
2. Antigen expression – IIF (nondif)
3. Gene expression – QRT-PCR (nondif + dif)
4. Gene imprinting (nondif)
5. Teratoma formation
6. Microbiological analysis (viruses, mycoplasmas,…)

- 63 hESC lines
- 17 laboratories
- 11 countries

CCTL9, CCTL12, CCTL14

Published
Nature Biotechnology
2007

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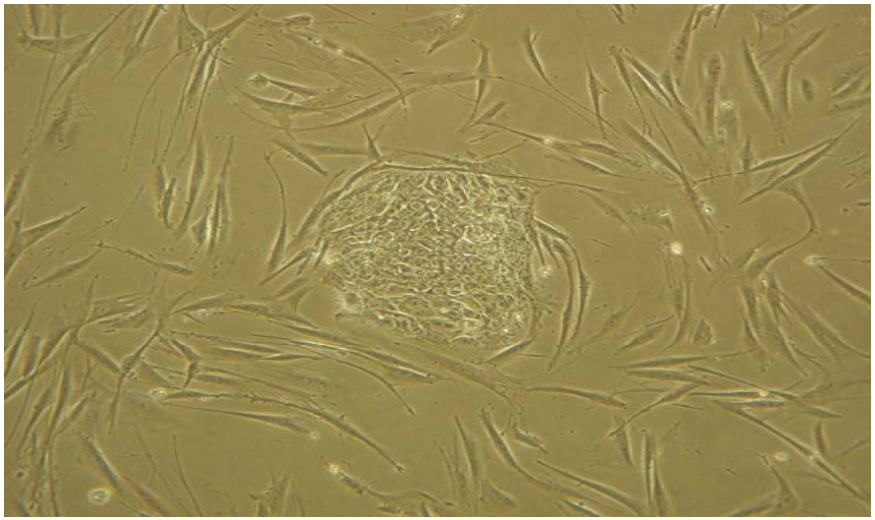
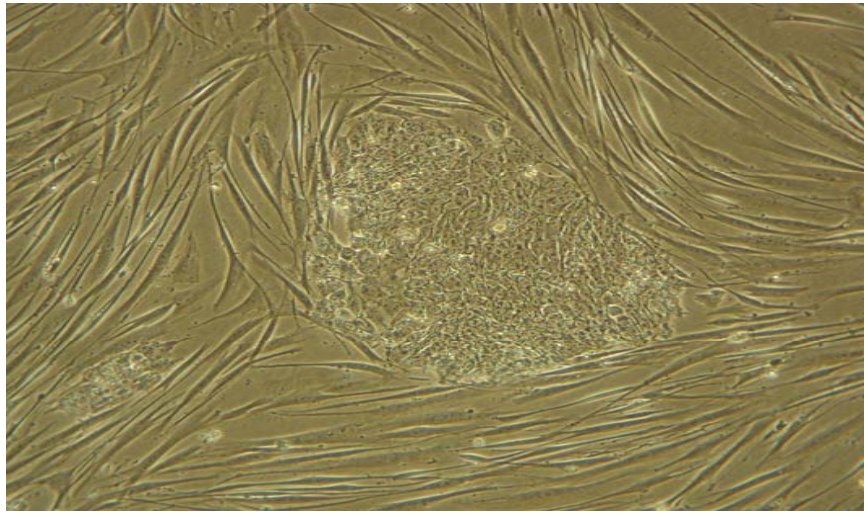
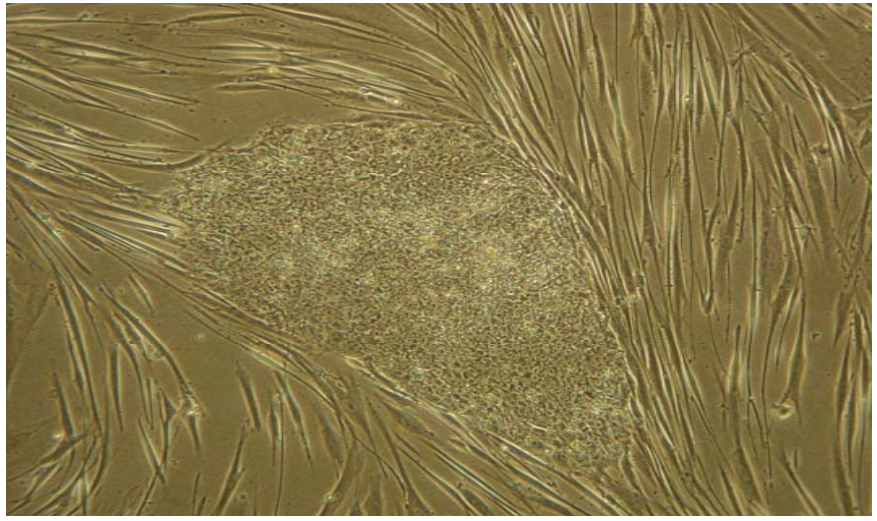
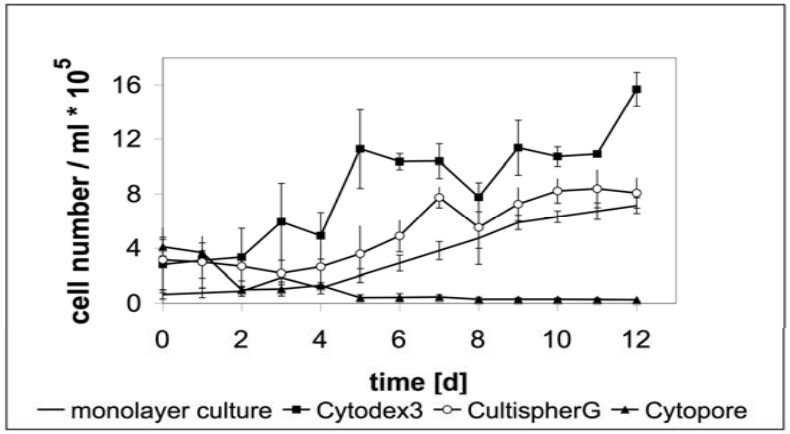


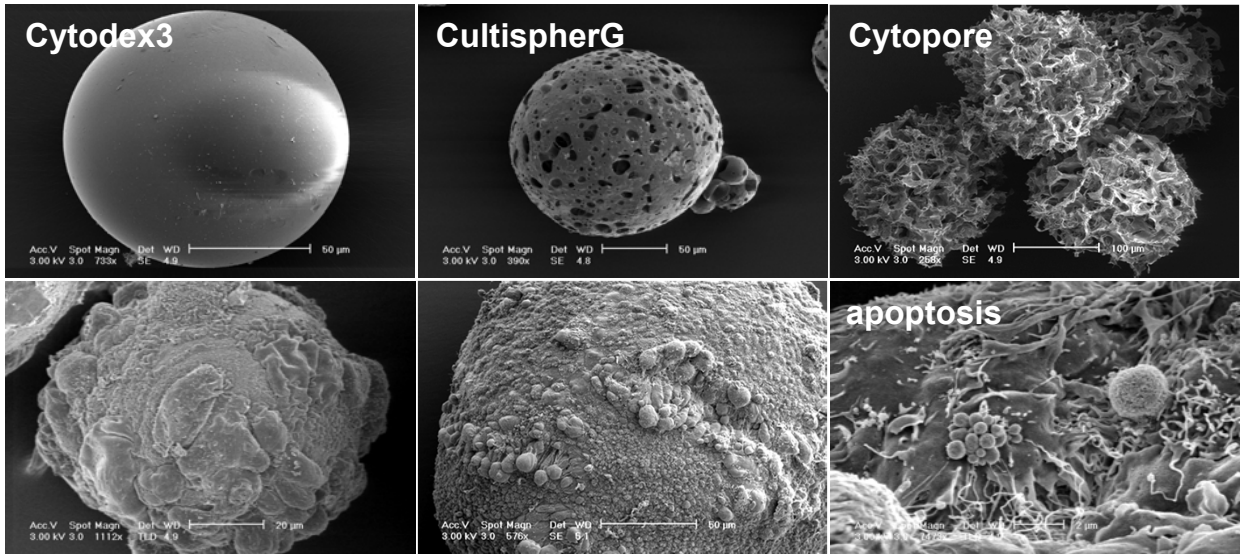
Human ES cells (line CCTL14) growing on feeder layer of human foreskin fibroblasts (line SCRC-1041)





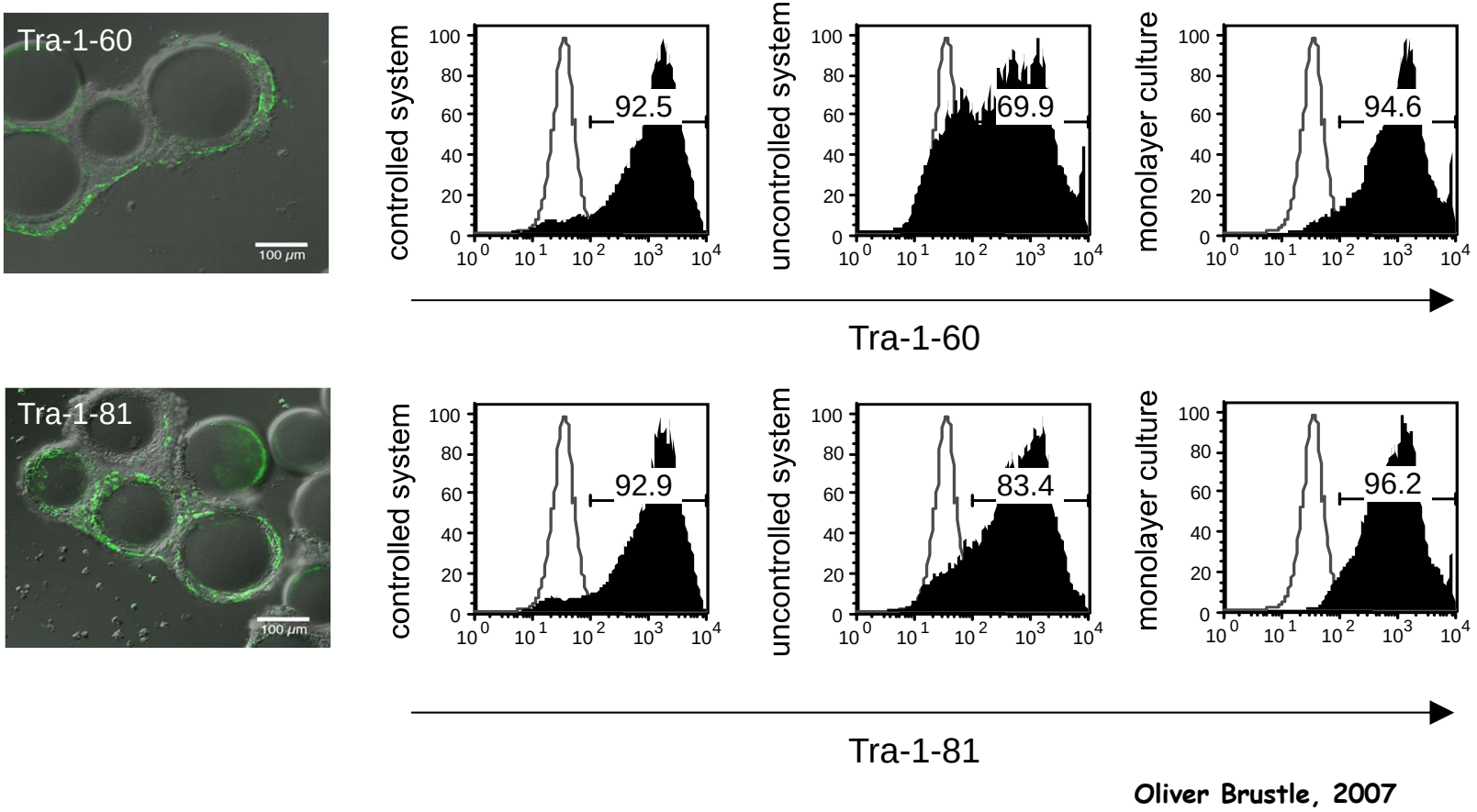
Culturing of human ES cells on corpuscular microcarriers in suspension – way of effective propagation ?





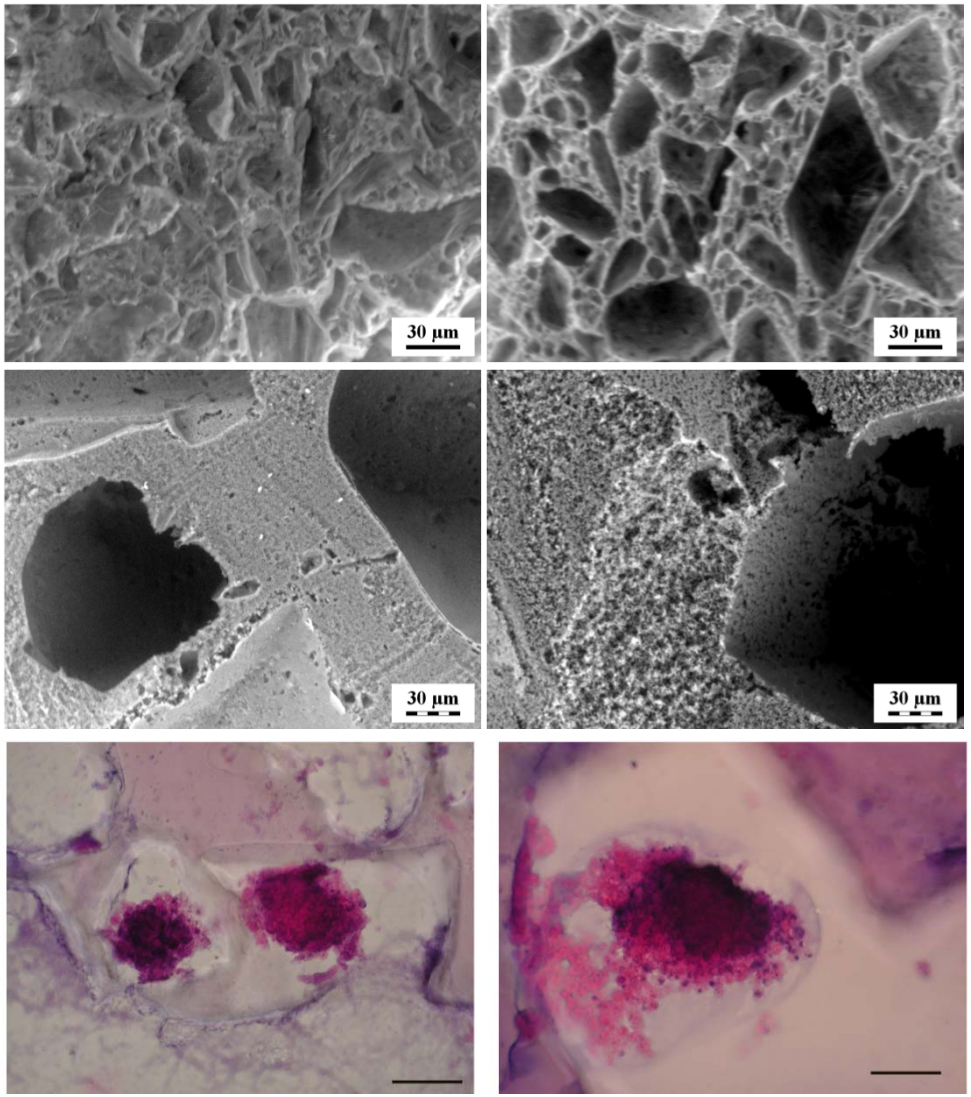
Oliver Brustle, 2007

Culturing of human ES cells on corpuscular microcarriers does not affect the expression of markers of pluripotency

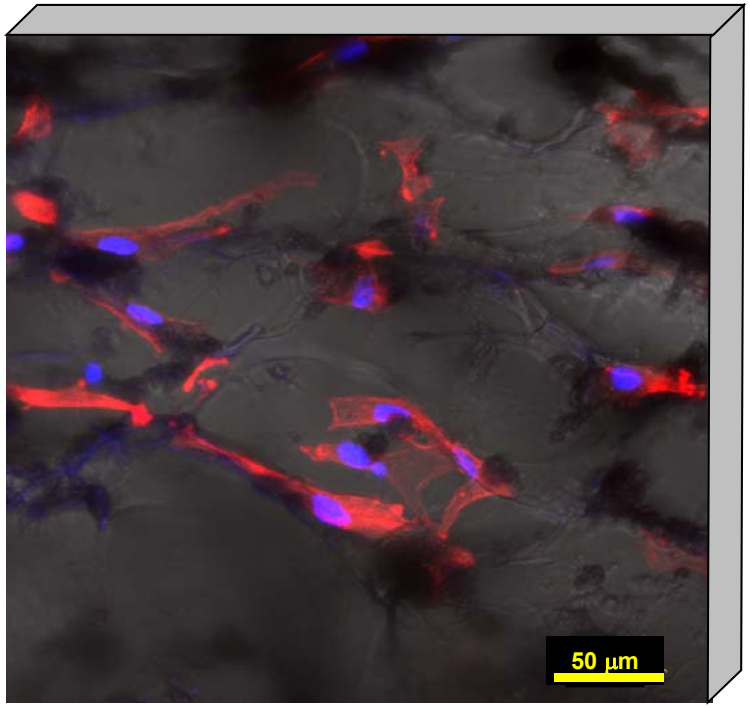


Oliver Brustle, 2007

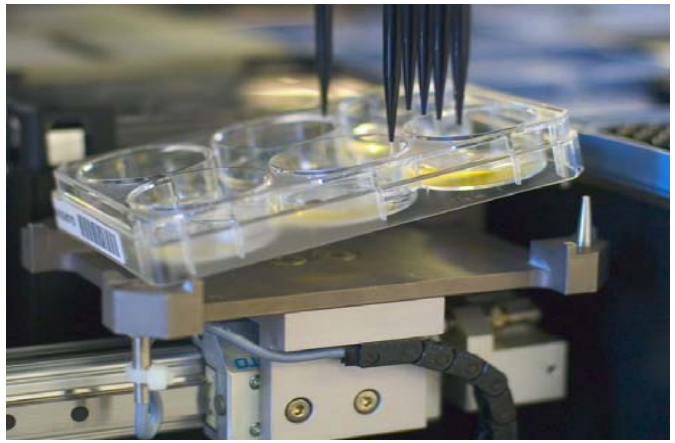
Hydrogels may function as carriers for human ES cells

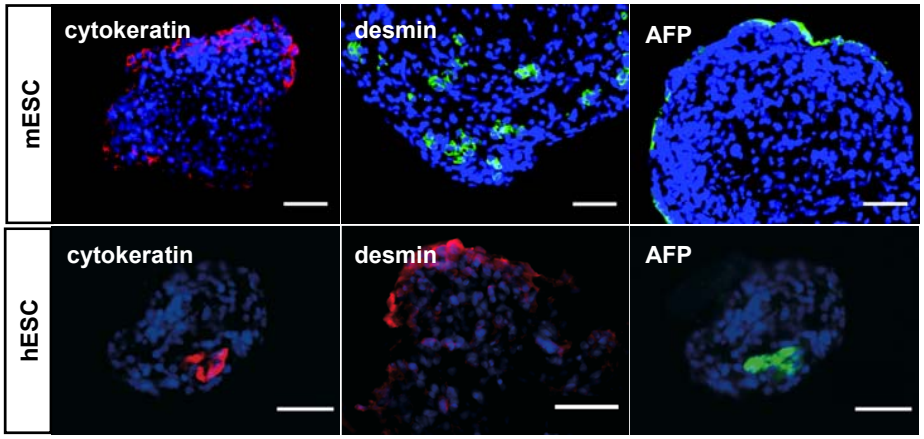


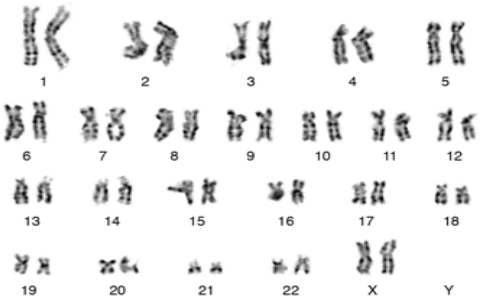
- Institute of macromolecular chemistry ASCR
- VUT Brno, Prof. Jančář



Cell^{host} system makes all the key steps in culturing of human ES cells automated

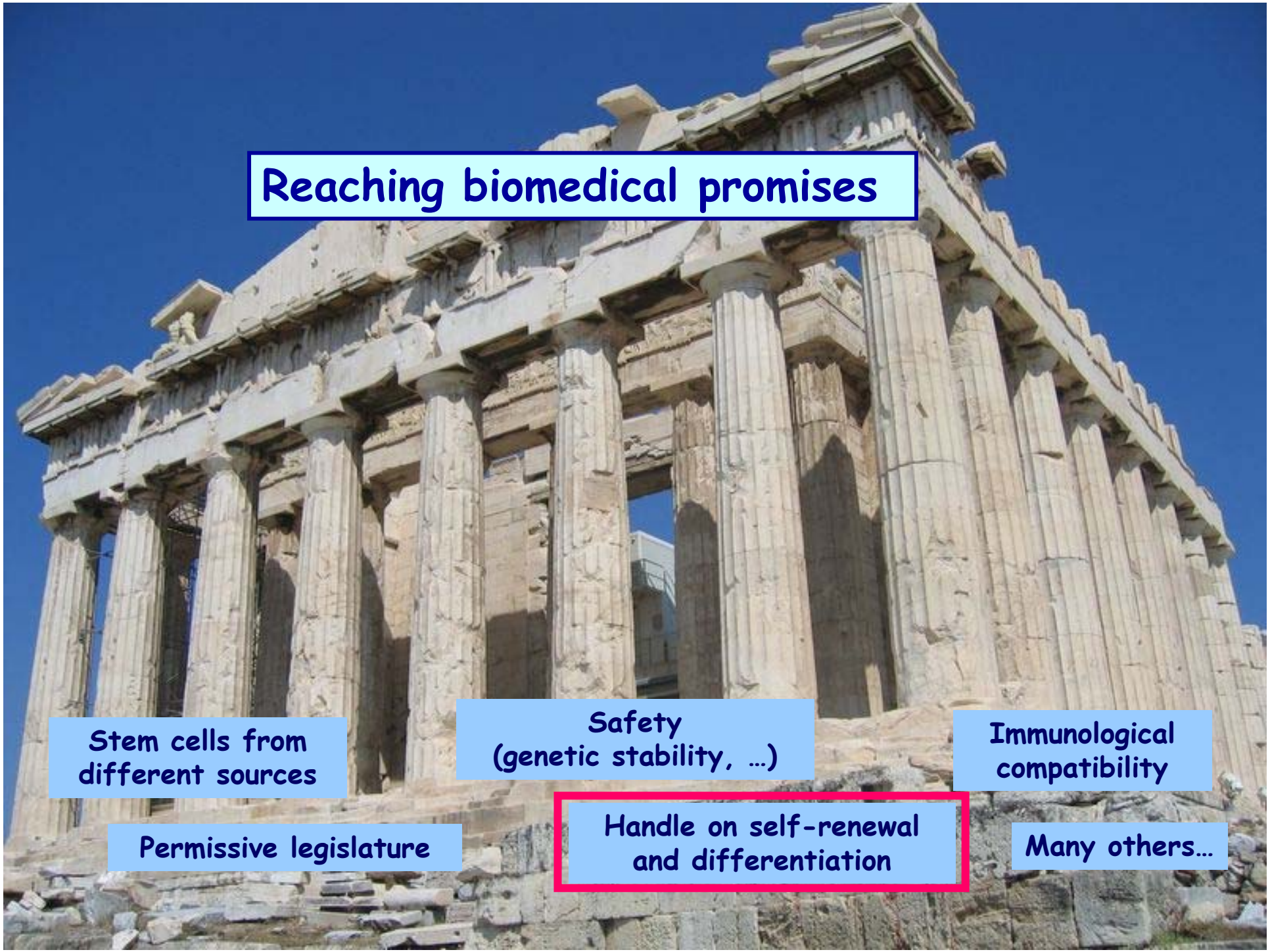






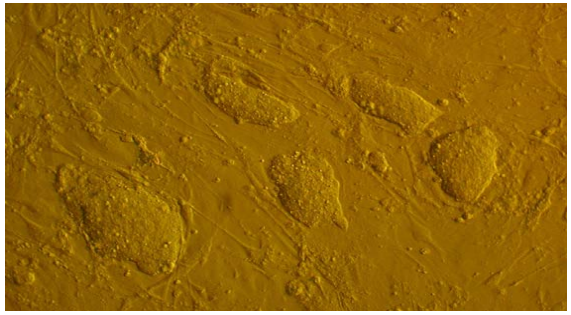
Oliver Brustle, 2007

University of Bonn


Reaching biomedical promises
Stem cells from different sources
Safety (genetic stability, …)
Immunological compatibility
Permissive legislature
Handle on self-renewal and differentiation
Many others…

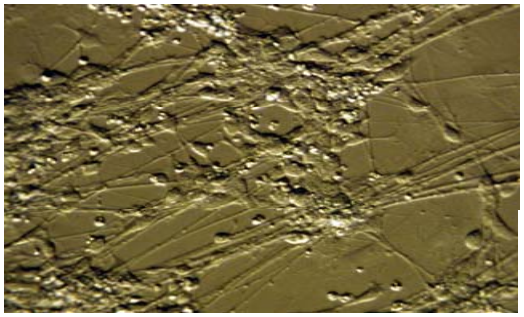
Pluripotency of PS cells

ectoderm	mezoderm	endoderm
brain spinal cord skin	blood muscles kidney	liver pancreas lungs intestine

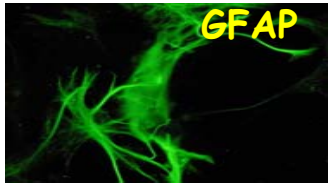


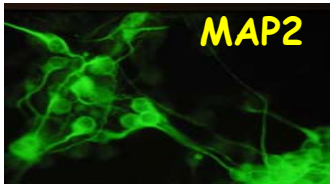
Nondifferentiated mES cells

Diferentiation
in vitro



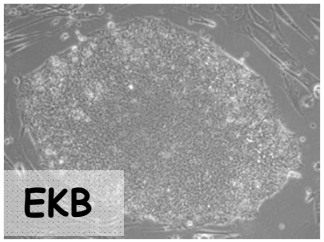
Differentiated mES cells
with the glial markers





How to achieve differentiation of ES cells into proper lineage?

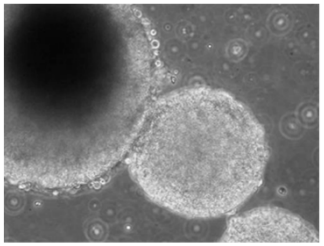
1) presence of „proper" growth factors (FGF2, EGF, IGF, RA, Noggin, …)
2) presence of proteins of extracellular matrix (colagen, laminin, fibronectin, …)
3) presence of interacting cell surface molecules (integrins, NCAM, ….)
4) structure / elasticity / size of the cell culture substrate
5) timing of all the treatments



Embryoid bodies (16 days)

→

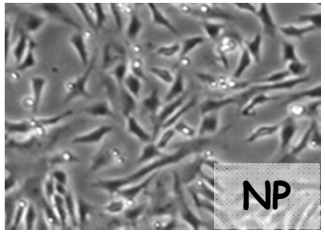
+ retinoic acid



Desintegration of EBs into single cells

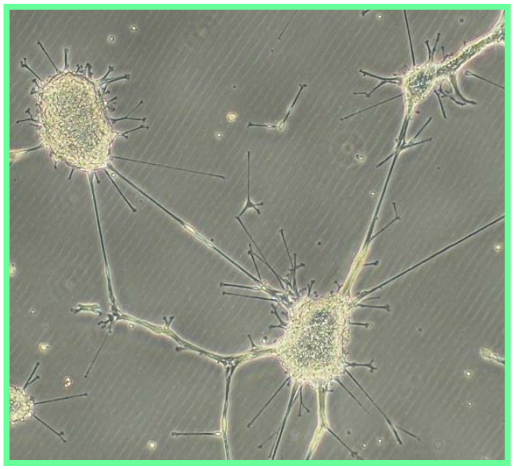
→

Culture on gelatin

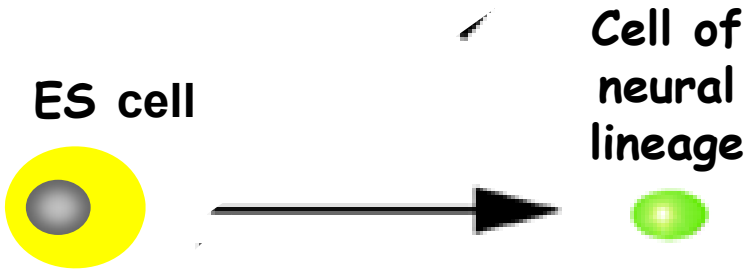


- Neurons, astrocytes, oligodendrocytes
- Pigmented epithelia of retina
- Cardiomyocytes
- Endotelial cells
- Insulin-producing cells
- Hematopoietic cells
- Immunocompetent cells
- Trophoblast cells
- Cells of respiratory epithelia
- Osteoblasts
- Hepatocytes
- Melanocytes
- Prostate cells

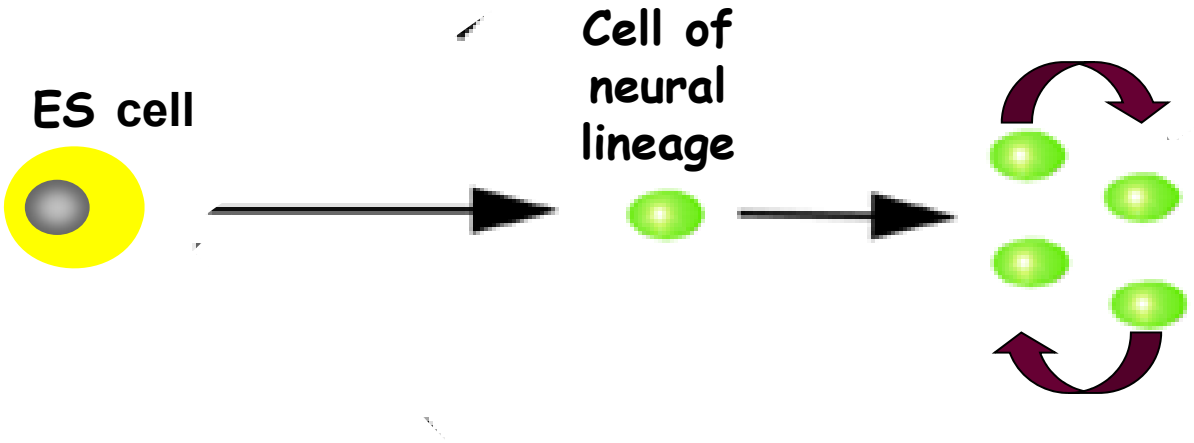
Terminal differentiation



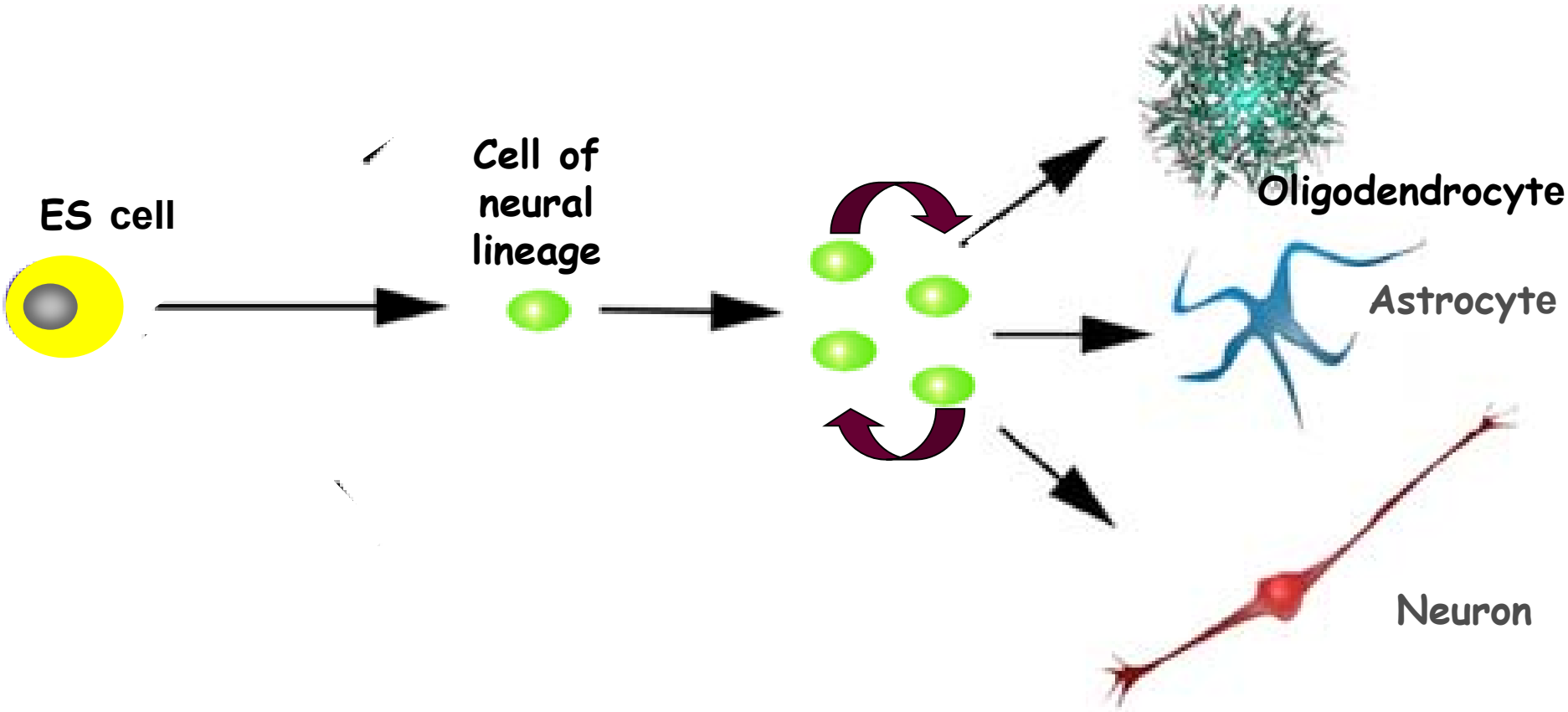
Initiation of differentiation into certain cell lineage – „commitment“

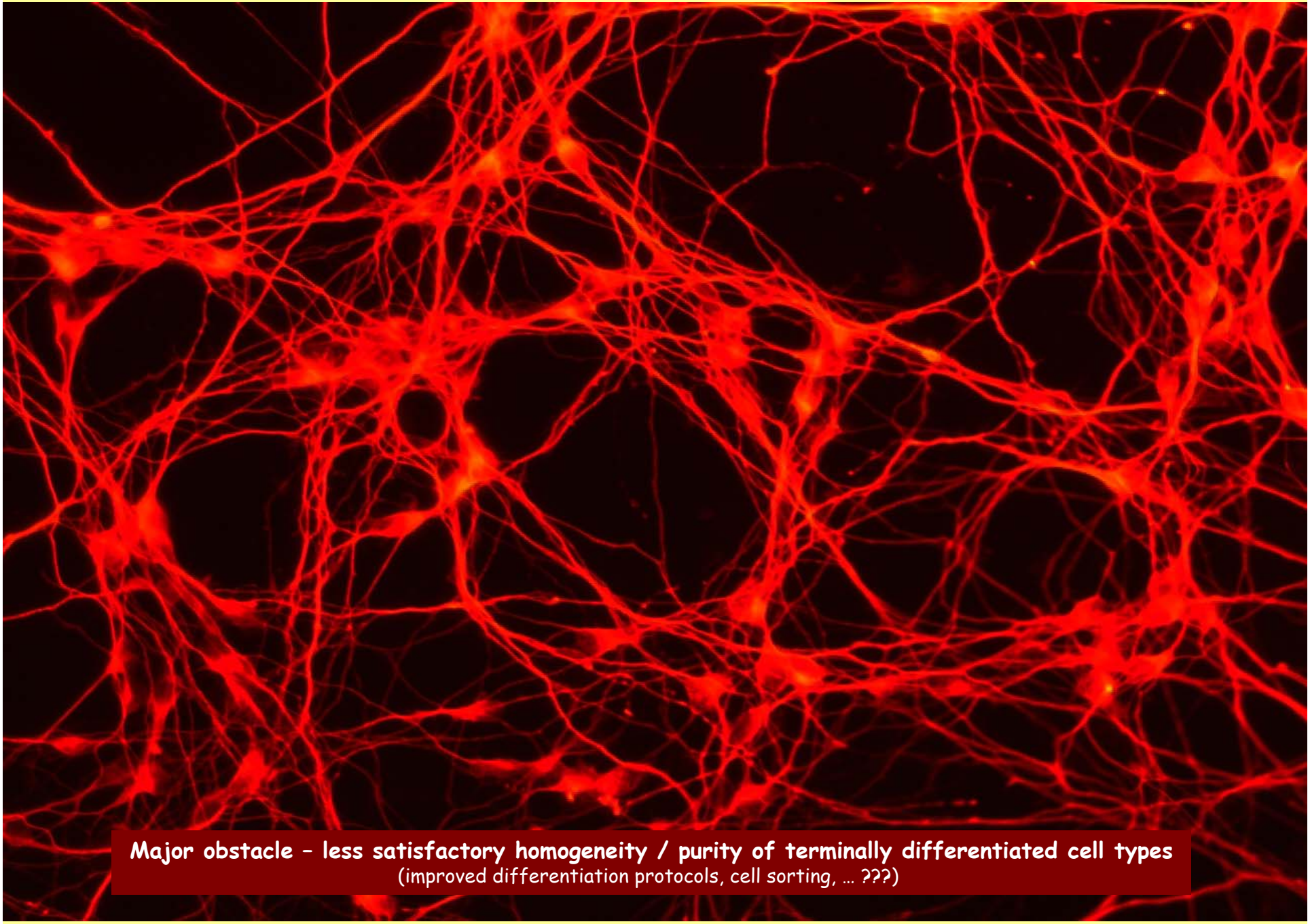


Expansion of progenitors



Specification and terminal differentiation into functional cell types



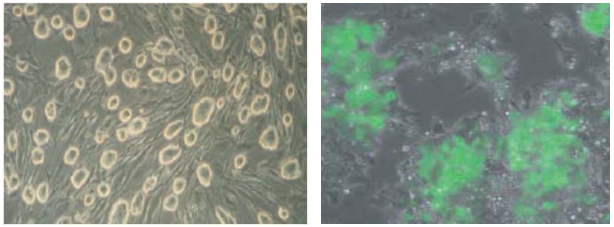


Major obstacle – less satisfactory homogeneity / purity of terminally differentiated cell types
(improved differentiation protocols, cell sorting, … ???)

In vitro culture can turn ESCs into female gametes

Science
AAAS

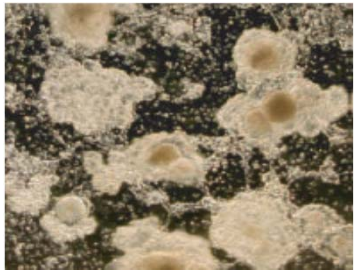
Derivation of Oocytes from Mouse Embryonic Stem Cells
Karin Hübner, *et al.*
Science **300**, 1251 (2003);
DOI: 10.1126/science.1083452

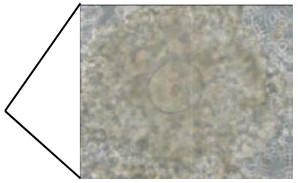


Oct-4-GFP reporter line

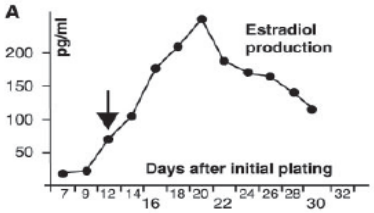
„FACS sorting"

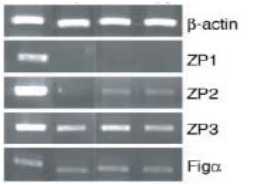
C-kit
VASA
SCP3

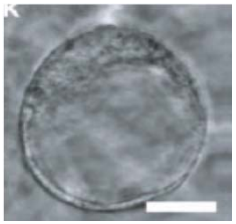


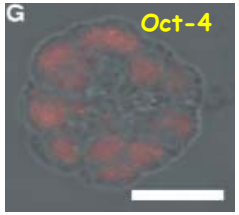


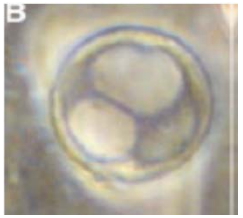
D22-26







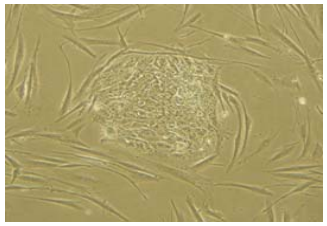




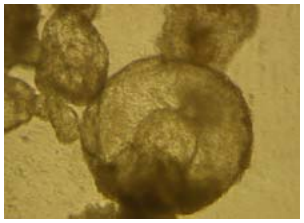
~D43

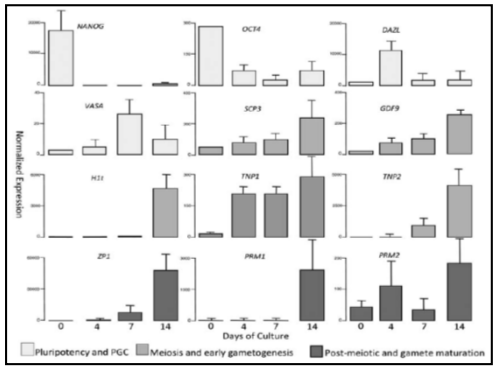
In vitro culture can turn hESCs into male and female gametes

- Shef1
- Shef3
- Shef4
- Shef5
- Shef6
- H7



+/-
- RA
- BMP
- Medium conditioned by neonatal mouse testes





human reproduction

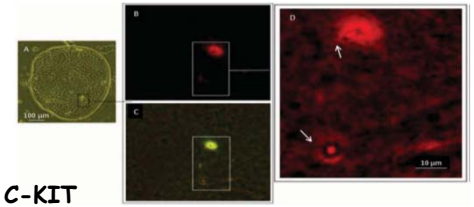
ORIGINAL ARTICLE *Reproductive biology*

In vitro post-meiotic germ cell development from human embryonic stem cells

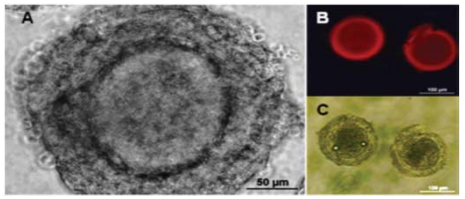
2009

B. Aflatoonian[1,2,5], L. Ruban[2,3], M. Jones[1], R. Aflatoonian[2,4], A. Fazeli[2], and H.D. Moore[1,2]

[1]Centre for Stem Cell Biology, Department of Biomedical Sciences, The University of Sheffield, Western Bank, Alfred Denny Building, Sheffield S10 2TN, UK [2]Academic Unit of Reproductive and Developmental Medicine, The University of Sheffield, Level 4, Jessop Wing, Tree Root Walk, Sheffield S10 2SF, UK [3]Present address: Department of Biochemical Engineering, University College London, Roberts Building, Torrington Place, London WC1E 7JE, UK [4]Present address: Reproductive and Biomedicine Group, Medical school, Iran University of Medical Sciences, Tehran 14155-5983, Iran



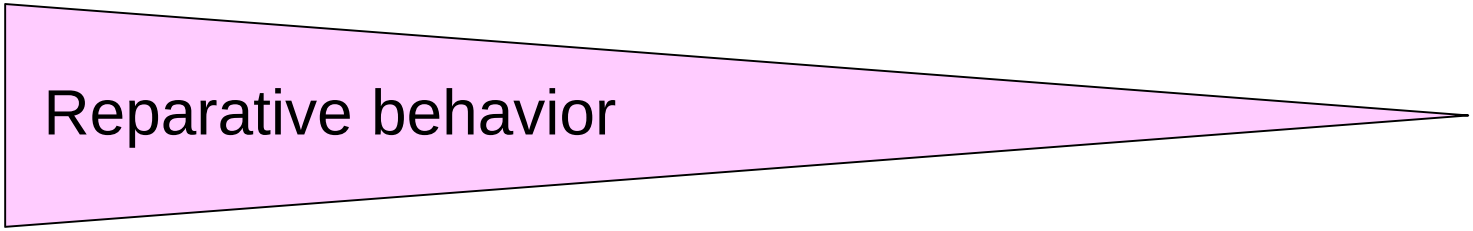
B) C-KIT
C) I-97 antigen
D) Cells with kondenzed chromatin and sperm-like morphology



Structures reminiscent to oocyte-granulosa cells complexes (ZP nondeveloped)

Last sentence: „Speculation on clinical applications for hESC-derived gametes is premature."

Stem cells can repair adult tisues/organs



- Constitutive high rate
- Defined hierarchy of stem/progenitor cells

Epidermis

Intestine

Blood

- Low steady-state turnover
- Robust repair after damage

Lung

Liver

Pancreas

- Inefficient
- Scaring instead of repair

Brain

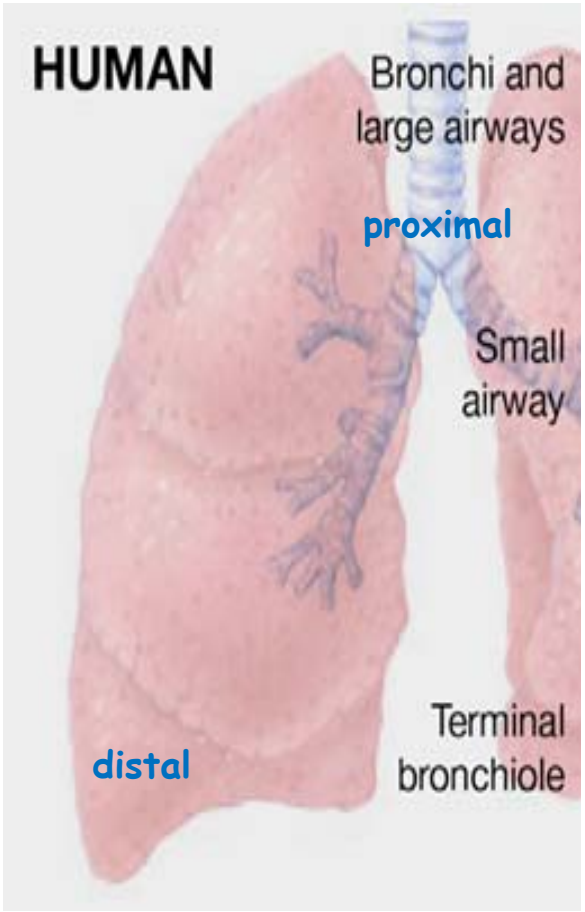
Heart

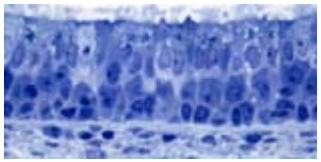
Lung structure

Anterior ventral foregut endoderm +

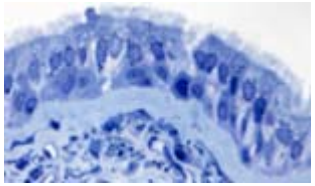
Mesoderm

Vascular smoth muscle
Airway smooth muscle
Cartilage
Myofibroblasts
Pericytes



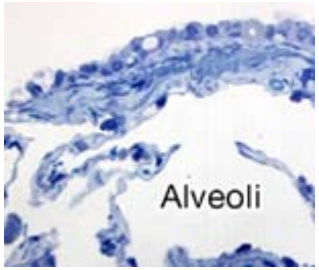


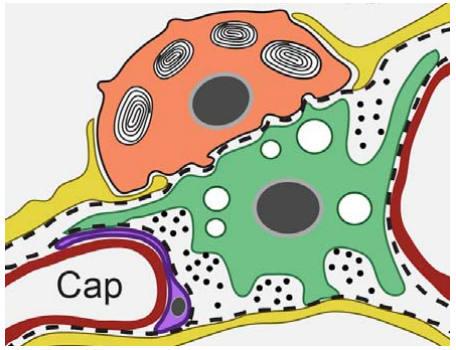
Ciliated cells
Goblet cells
Serous cells
Neuroendocrine cells
Basal cells



Clara cells
Club cells
Ciliated cells
Basal cells

Pneumocytes type I





Pneumoctes type II

More than 40 cell lineages identified in lungs !!!

Lung diseases potentially treatable by cell therapies.

Respiratory diseases are the **third leading cause of death** in the industrialized world.

Lung replacement is often the only solution.

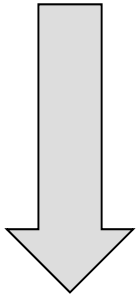
Lung disease	Affected components	Therapeutic target
Respiratory distress syndrome	Alveolar epithelium Capillary endothelium	Epithelia and endothelia regeneration
Asthma	Epithelium Myofibroblast Airway smooth muscle	Inhibition of inflamation Inhibition of airway remodeling, Inhibition of muscle heperplasia
Bronchopulmonary dysplasia	Alveolar epithelium Capillary endothelium Interstitial fibroblasts	Inhbition of inflamation Regeneration of alveolar septa and epithelium
Cystic fibrosis	Airway epithelium	Delivery of CFTR (cystic fibrosis conductance regulator)
Chronic obstructive pulmonary diseases (emphysema)	Alveolar epithelium Capillary endothelium Interstitial fibroblasts	Generate 3D alveolar structure
Bronchiolitis obliterans	Airway epithelium	Regeneration of epithelia
Cancer	All components	Complete replacement of 3D structure

and others

Therapeutic strategies

Acute alveolar damage

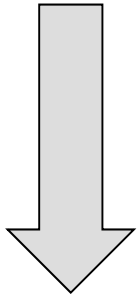
- inhalation injury
- blast injury



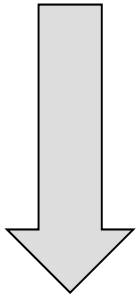
Activation of healing potential of resident progenitors

Chronic lung damage

- Chronic obstructive pulm. disease
- Fibrosis
- Bronchopulmonary displasia
- and others

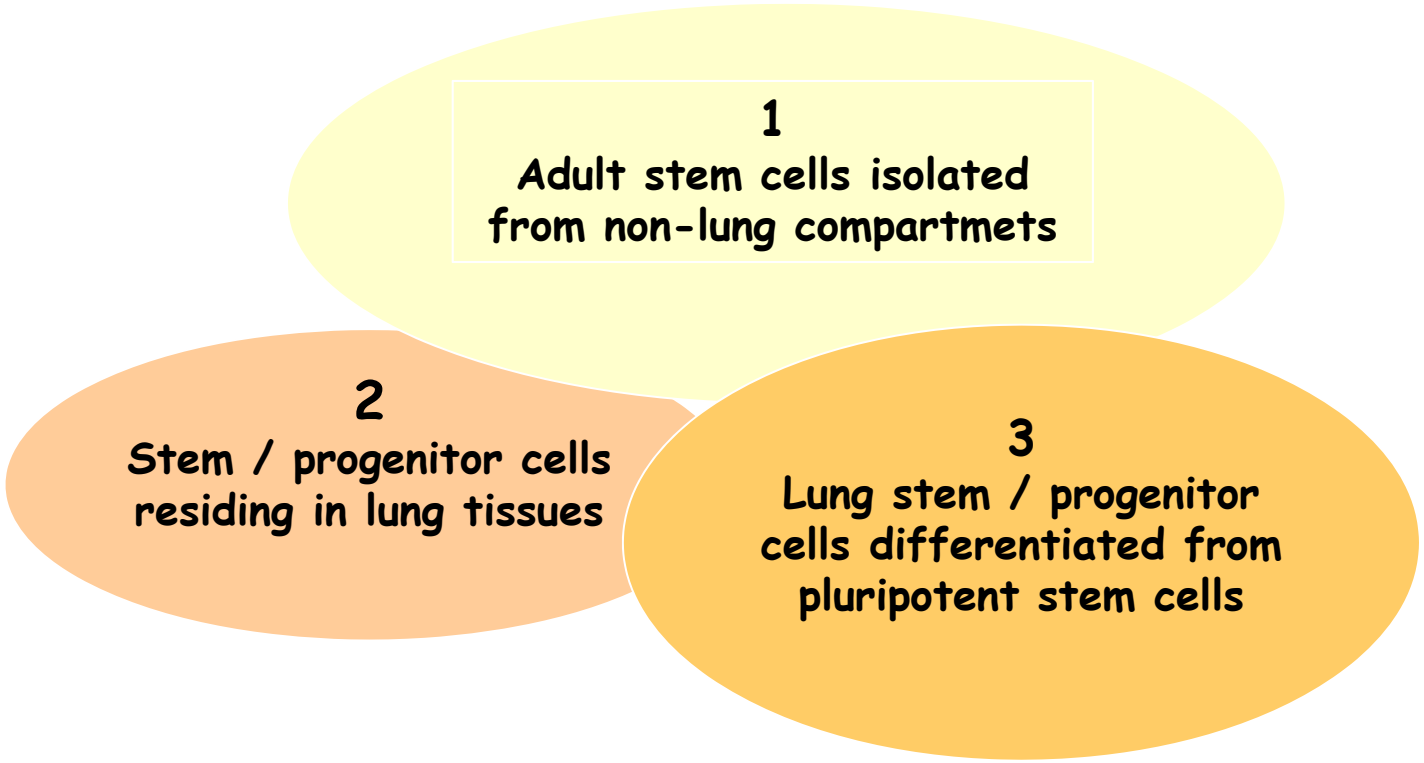


Cellular therapy



Lung engineering

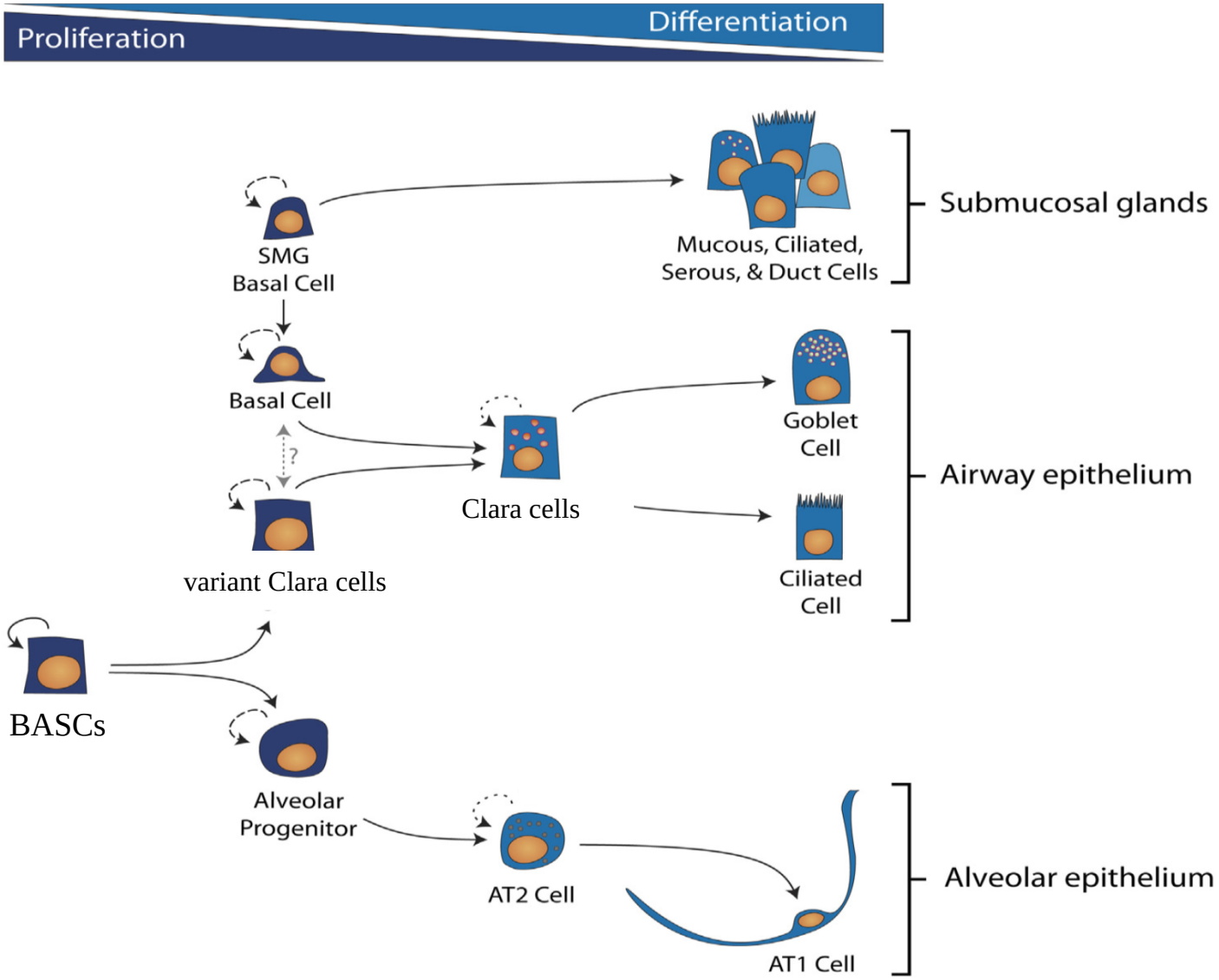
What cell sources we may consider?



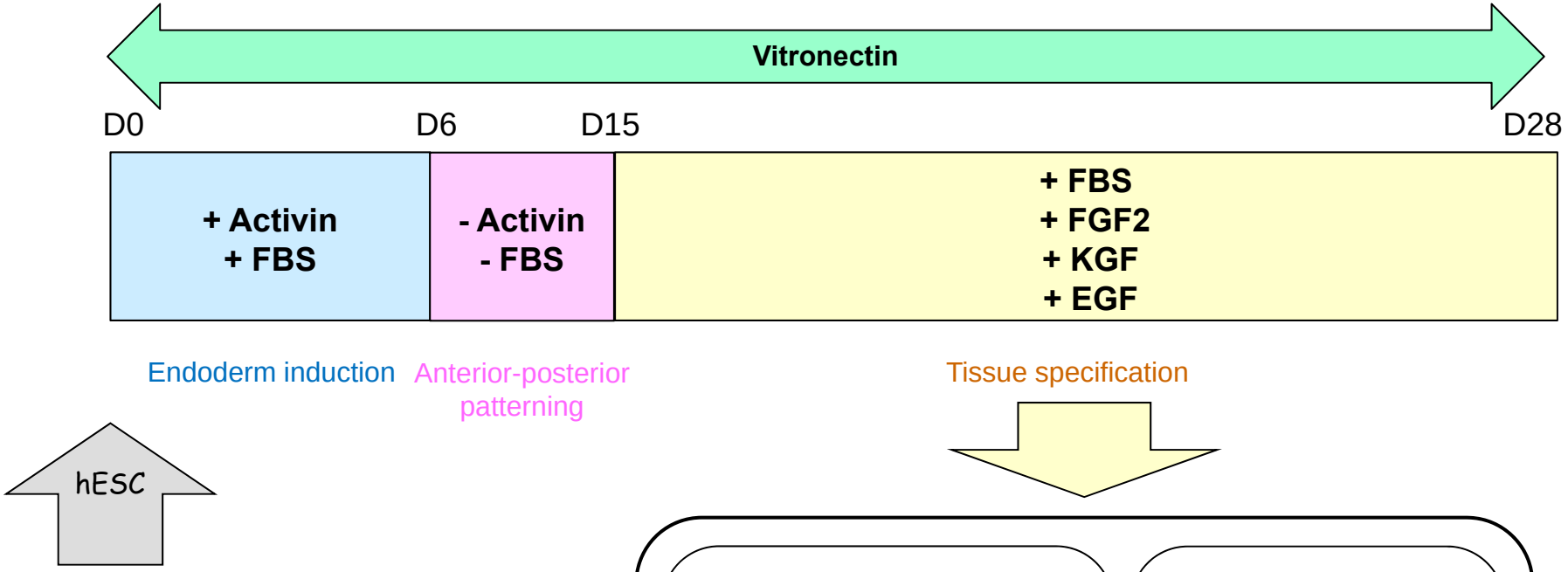
Stem / progenitor cells residing in lung tissues

Anas Rabatta

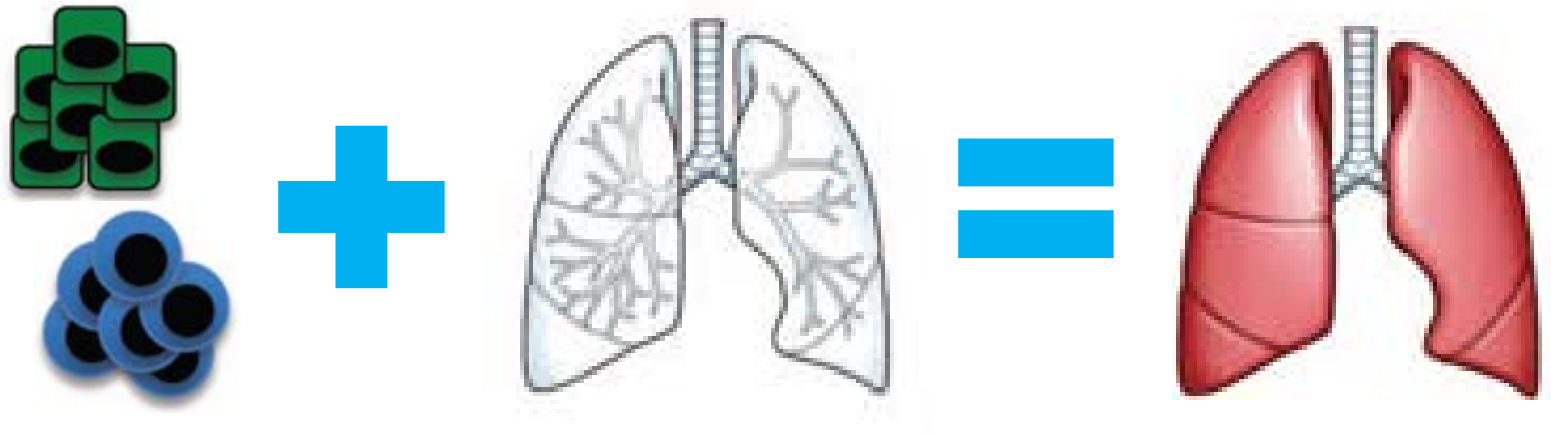
Zuzana Koledová



Direct differentiation of pluripotent SC into airway epithelia



From individual cells to 3D organ-resembling structures



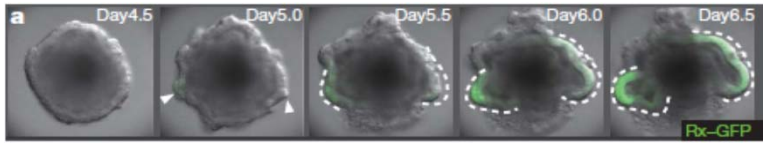
ESC can give rise to highly organized organ-like structures.

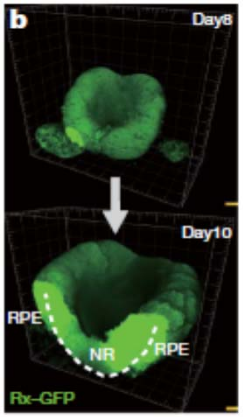
3D culture (EB)
+ integrins
+ laminin
+ entactin
+ Nodal

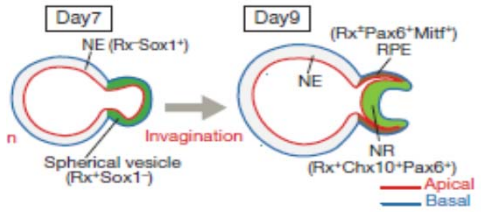
ARTICLE April 2011
doi:10.1038/nature09941

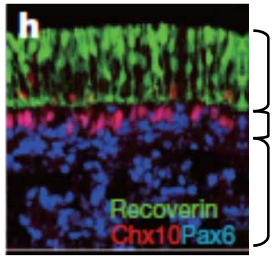
Self-organizing optic-cup morphogenesis in three-dimensional culture

Mototsugu Eiraku[1,2], Nozomu Takata[1], Hiroki Ishibashi[3], Masako Kawada[1], Eriko Sakakura[1,2], Satoru Okuda[3], Kiyotoshi Sekiguchi[4], Taiji Adachi[3,5] & Yoshiki Sasai[1,2]









Internal nuclear layer
External nuclear layer
Ganglion cells

D24

Even induced pluriptent SC go to the clinic.

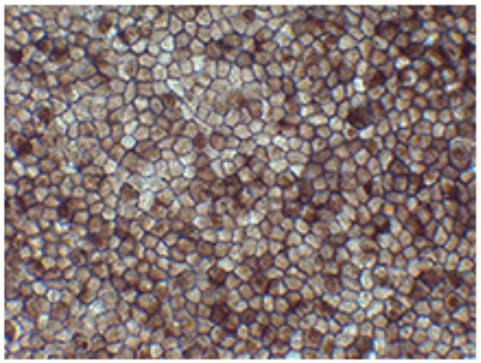
Press Release

July 30, 2013

Pilot clinical study into iPS cell therapy for eye disease starts in Japan

RIKEN is pleased to announce the launch of a pilot study to assess the safety and feasibility of the transplantation of autologous induced pluripotent stem cell (iPSC)-derived retinal pigment epithelium (RPE) cell sheets in patients with exudative (wet-type) age-related macular degeneration.

This study, led by Masayo Takahashi M.D., Ph. D. of the Laboratory for Retinal Regeneration, RIKEN Center for Developmental Biology, and conducted in collaboration with the Institute for Biomedical Research and Innovation with support from the Kobe City Medical Center General Hospital, has been approved to proceed following review by the Ministry of Health, Labour and Welfare and is scheduled to open patient recruitment on August 1, 2013.



Retinal Pigment Epithelium (RPE) cells derived from human iPS Cells

Age-related macular degeneration is the most common cause of visual impairment in the elderly, and affects up to 1% of people over 50 years of age in Japan. Wet-type AMD is characterized by progressive damage to the retinal pigment epithelium, a protective layer of non-neural cells located adjacent to the photoreceptors at the back of the eye, due to leakage caused by neovascularization.

Genetic changes develop in self-renewing hESC.

2010

OPEN ACCESS Freely available online

PLoS one

A Teratocarcinoma-Like Human Embryonic Stem Cell (hESC) Line and Four hESC Lines Reveal Potentially Oncogenic Genomic Changes

Outi Hovatta[1]*, Marisa Jaconi[2], Virpi Töhönen[3], Frédérique Béna[4], Stefania Gimelli[4], Alexis
Frida Holm[1], Stefan Wyder[5], Evgeny M. Zdobnov[5], Olivier Irion[6], Peter W. Andrews[7], Stylia
Antonarakis[4], Marco Zucchelli[3], Juha Kere[3☯], Anis Feki[6☯]

Aging Cell

October 2011

Increased dosage of tumor suppressors limits the tumorigenicity of iPS cells without affecting their pluripotency

Sergio Menendez[1], Suzanne Camus[1,§], Aida Herreria[1,§], Ida Paramonov[1], Laura Batlle Morera[1], Manuel Collado[2], Vlad Pekarik[1], Iago Maceda[1], Michael Edel[1], Antonella Consiglio[1,‡], Adriana Sanchez[1,‡], Han Li[2], Manuel Serrano[2], Juan Carlos Izpisua Belmonte[1,3]

DOI: 10.1111/j.1474-9726.2011.00754.x

Issue

Aging Cell

Accepted Article (Accepted, unedited articles published online for future issues)

PERSPE

nature biotechnology

2007

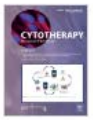
Adaptation to culture of human embryonic stem cells and oncogenesis *in vivo*

Duncan E C Baker[1,4], Neil J Harrison[2,4], Edna Maltby[1], Kath Smith[1], Harry D Moore[2], Pamela J Shaw[3], Paul R Heath[3], Hazel Holden[3] & Peter W Andrews[2]

…. and also in adult stem cells.

Cytotherapy

Volume 15, Issue 11, November 2013, Pages 1352–1361



Original paper

Culture expansion induces non-tumorigenic aneuploidy in adipose tissue-derived mesenchymal stromal cells

Marieke Roemeling-van Rhijn[1], Annelies de Klein[2], Hannie Douben[2], Qiuwei Pan[3], Luc J.W. van der Laan[4], Jan N.M. Ijzermans[4], Michiel G.H. Betjes[1], Carla C. Baan[1], Willem Weimar[1], Martin J. Hoogduijn[1]

STEM CELLS AND DEVELOPMENT
Volume 00, Number 00, 2014

DOI: 10.1089/scd.2014.0137

ORIGINAL RESEARCH REPORT

Asymmetric Aneuploidy in Mesenchymal Stromal Cells Detected by In Situ Karyotyping and Fluorescence In Situ Hybridization: Suggestions for Reference Values for Stem Cells

Seon Young Kim,[1] Kyongok Im,[2] Si Nae Park,[2] Jiseok Kwon,[2] Jung-Ah Kim,[1] Qute Choi,[1] Sang Mee Hwang,[1,3] Sung-Hee Han,[4] Sunghoon Kwon,[5] Il-Hoan Oh,[6] and Dong Soon Lee[1,2]

Expert Opinion on Biological Therapy, Ahead of Print : Pages 1-18

Placental mesenchymal stem cells of fetal origin deposit epigenetic alterations during long-term culture under serum-free condition

Yongzhao Zhu, Xumei Song, Jian Wang, Yukui Li, Yinxue Yang, Tingting Yang, Haibin Ma, Libin Wang, Guangyi Zhang, William C Cho, Xiaoming Liu, Jun Wei

(doi: 10.1517/14712598.2015.960837)

Cytotherapy

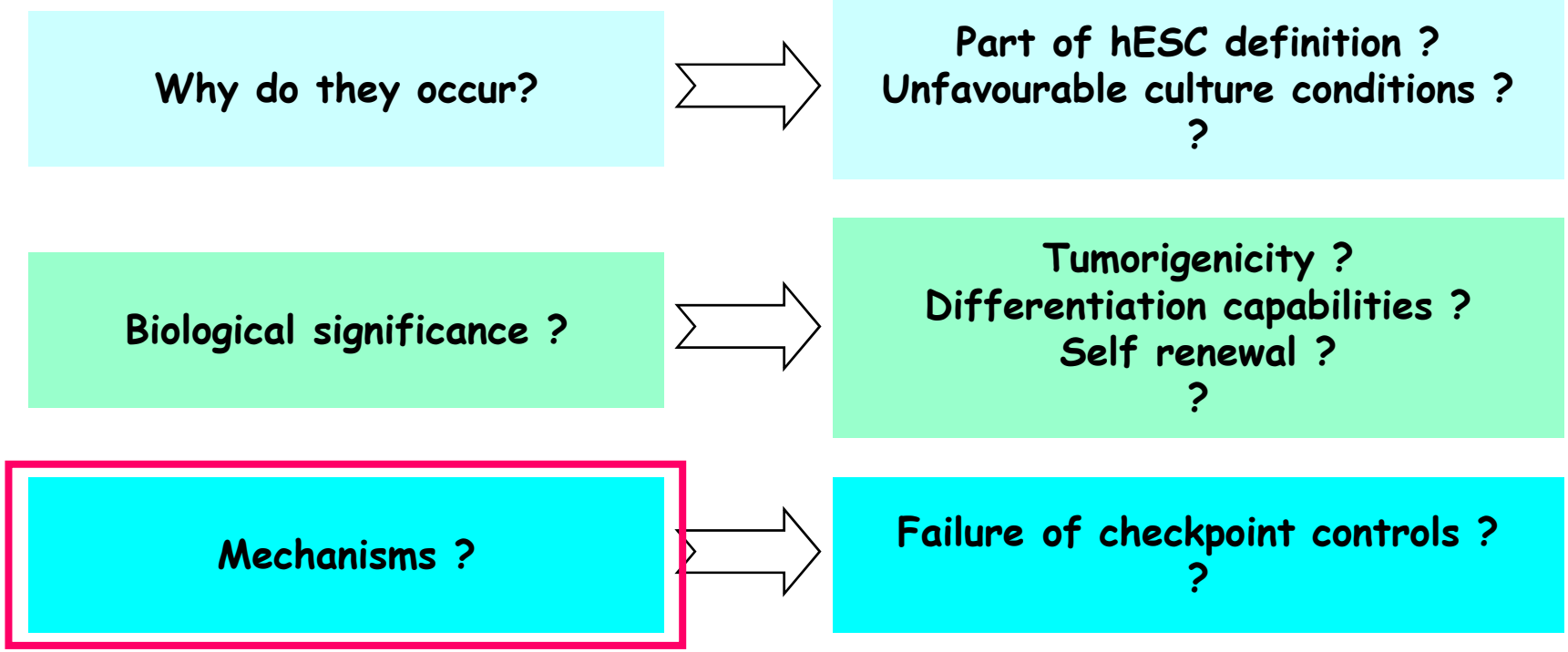
Volume 15, Issue 11, November 2013, Pages 1362–1373

Original paper

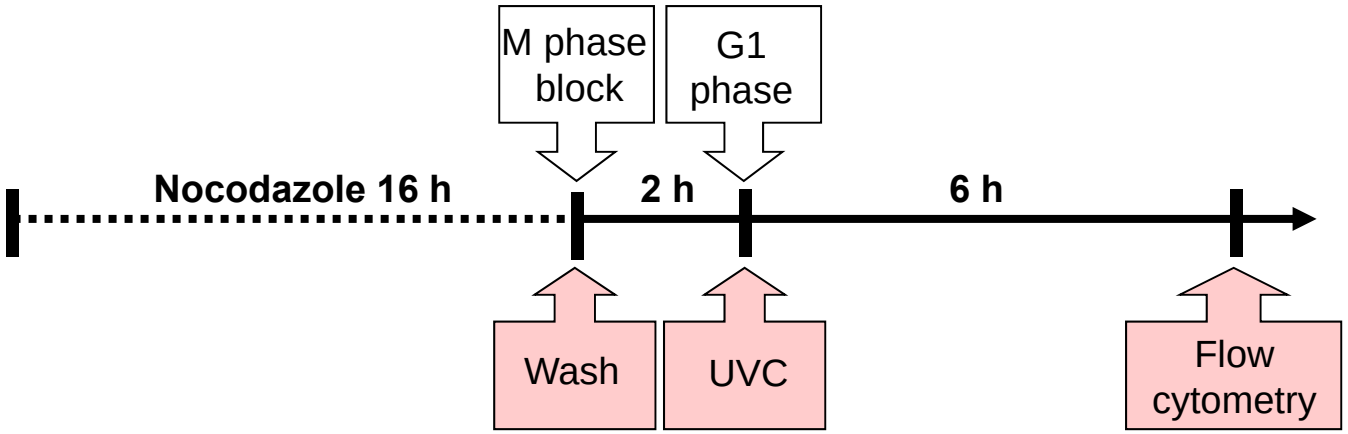
Genomic alterations in human umbilical cord–derived mesenchymal stromal cells call for stringent quality control before any possible therapeutic approach

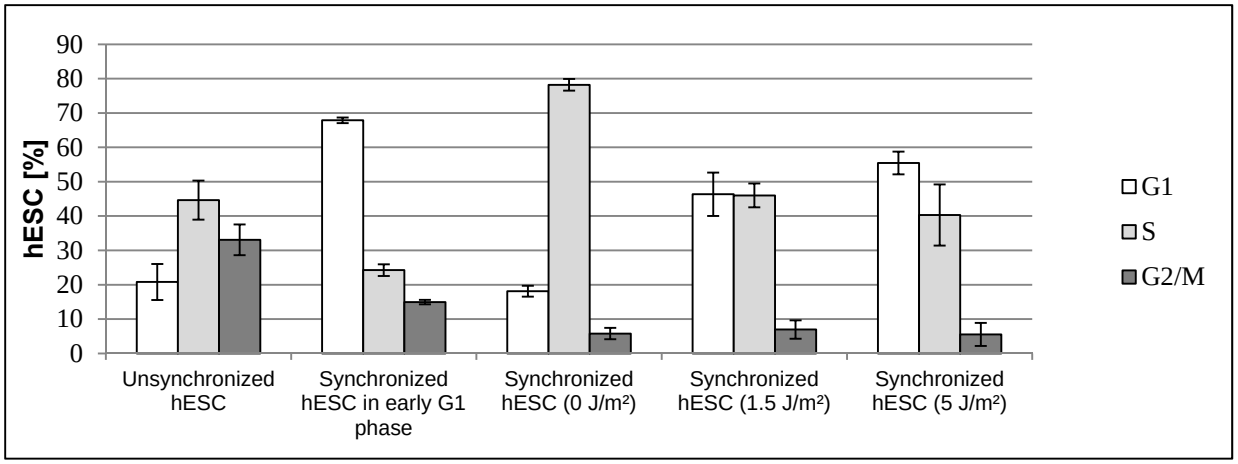
Alessandro Borghesi[1, 2, *], Maria Antonietta Avanzini[3, *], Francesca Novara[4, *], Melissa Mantelli[3], Elisa Lenta[3, 5], Valentina Achille[3], Rosa Maria Cerbo[1], Chryssoula Tzialla[1], Stefania Longo[1], Annalisa De Silvestri[6], Luc J.I. Zimmermann[7], Paolo Manzoni[8], Marco Zecca[9], Arsenio Spinillo[10], Rita Maccario[3, *], Orsetta Zuffardi[4, 11, *], Mauro Stronati[1, 2, *]

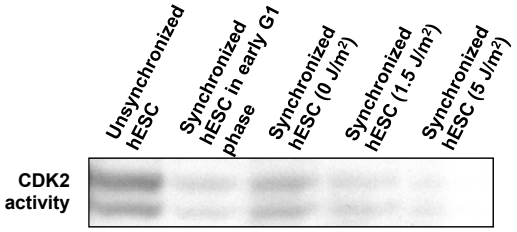
Alterations to the genome of hESC
Questions to be answered



G1 delay associated with inhibited CDK2 is produced by UVC irradiation of hESC.

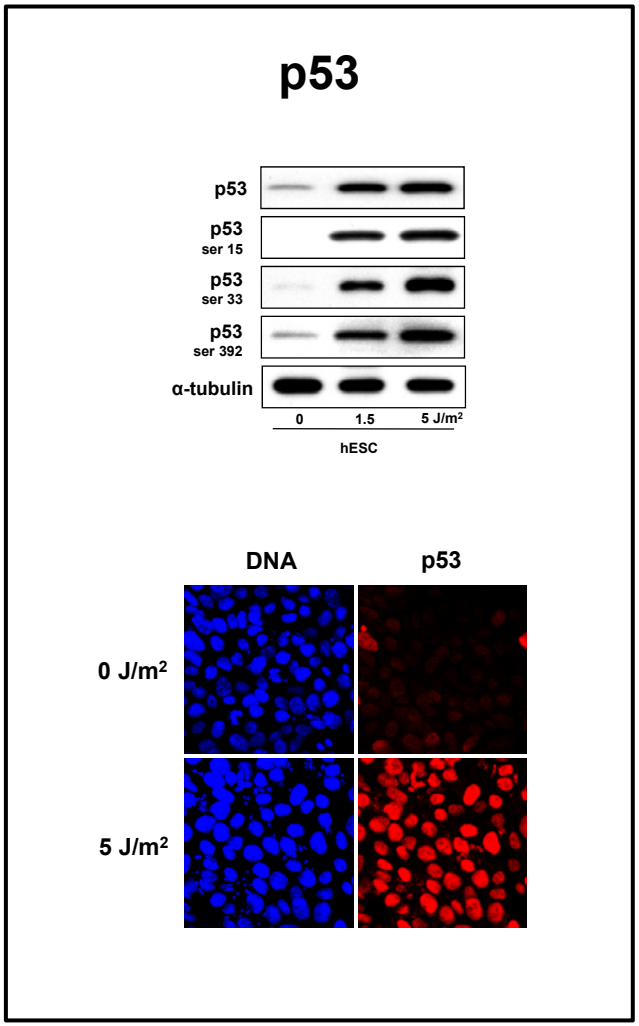


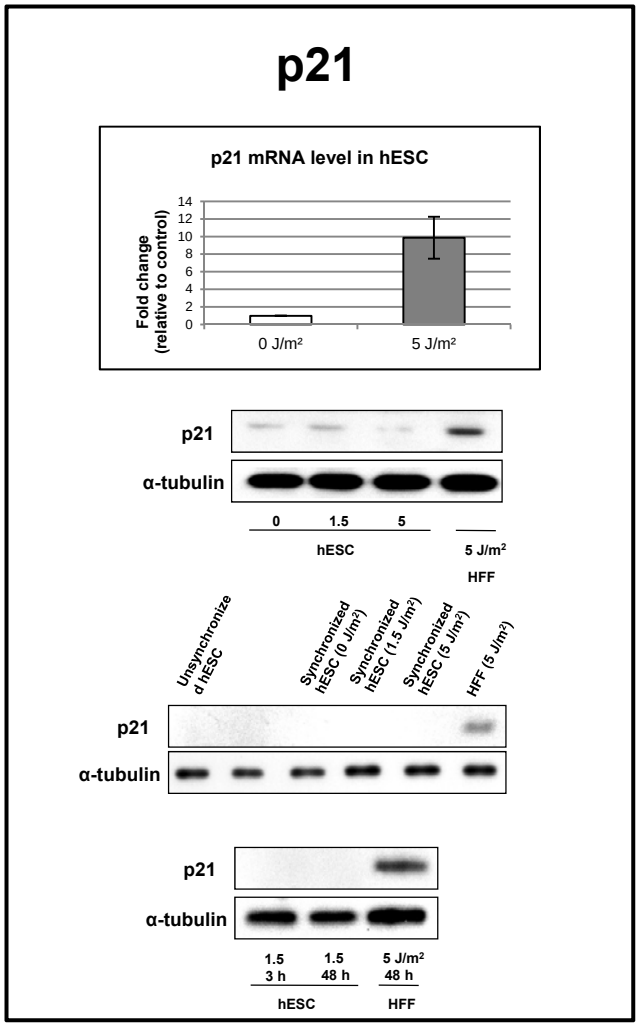




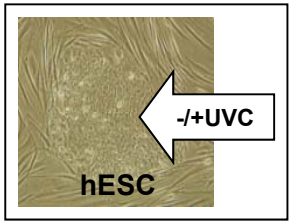
Barta et al., Stem Cells, 2011

UVC-induced damage does not lead to fruitful activation of p53–p21 axis in hESC.



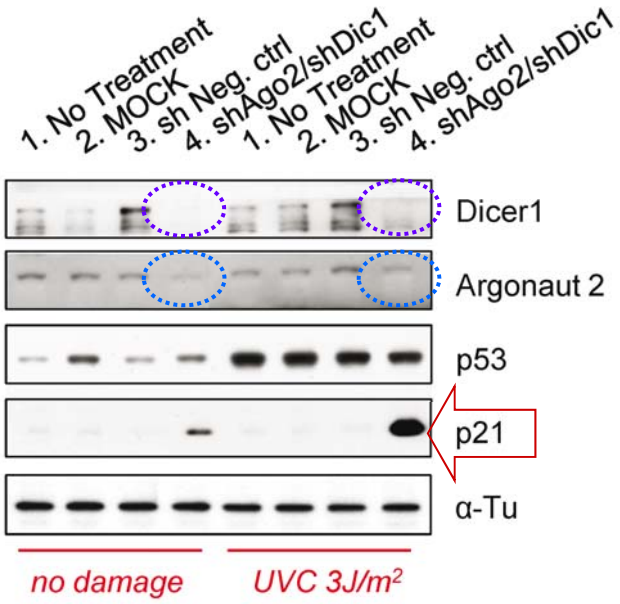


Barta et al.,
Stem Cells, 2011

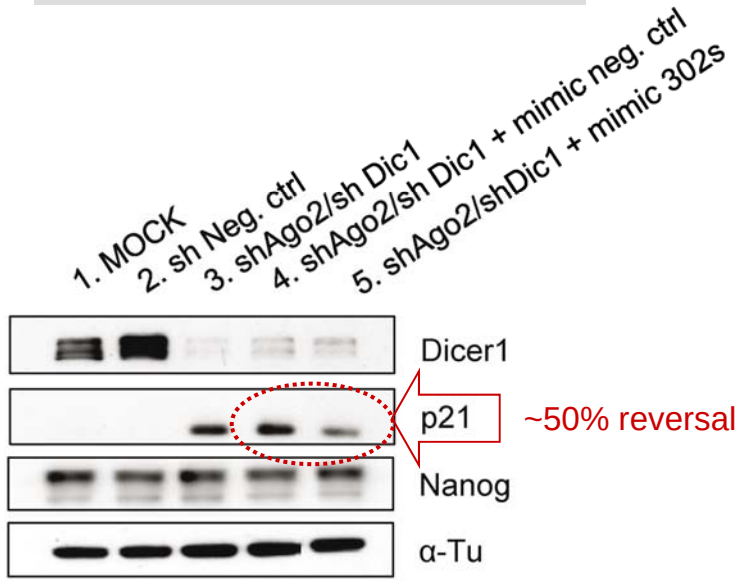


Members of miR-302 family regulate p21 in hESC.

+ Short-hairpin RNAs against Dicer 1 and Argonaut 2

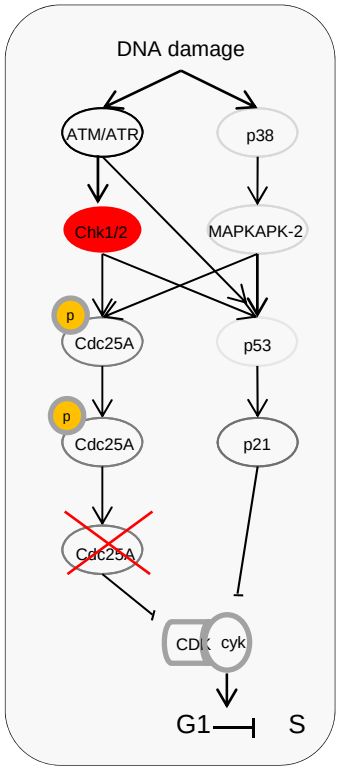


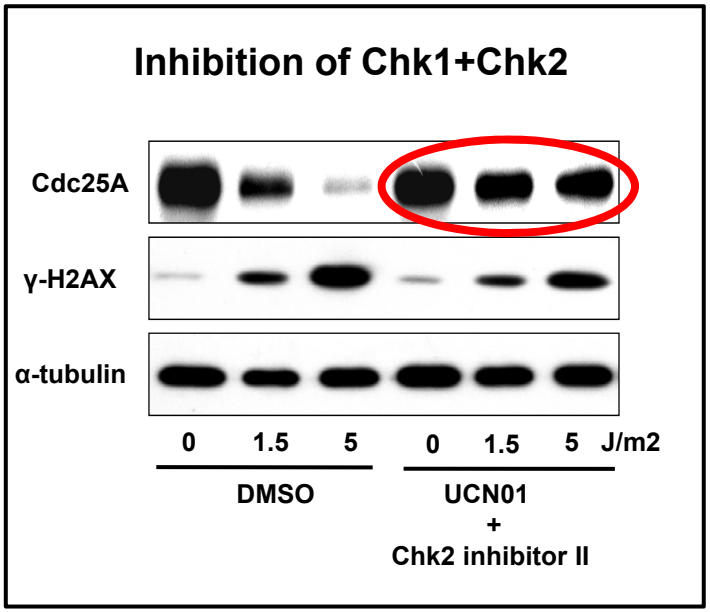
+ microRNA mimics of four members of miR-3O2 family

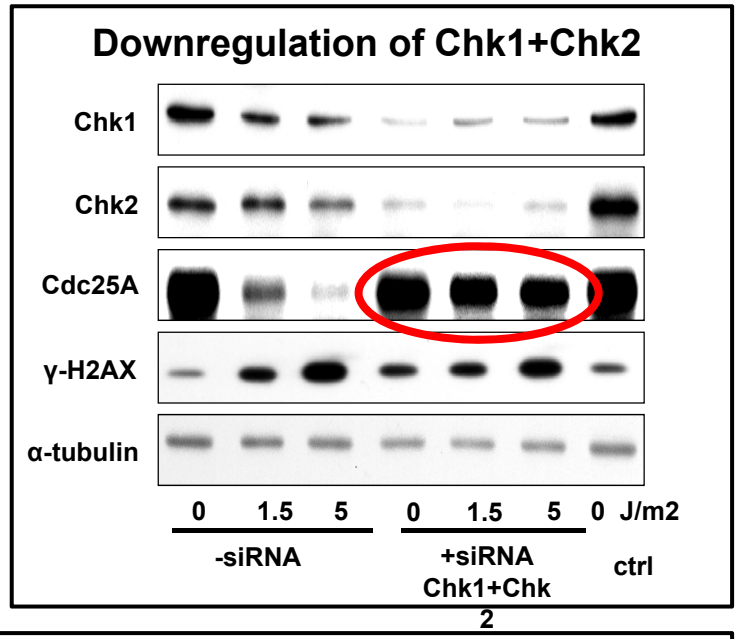


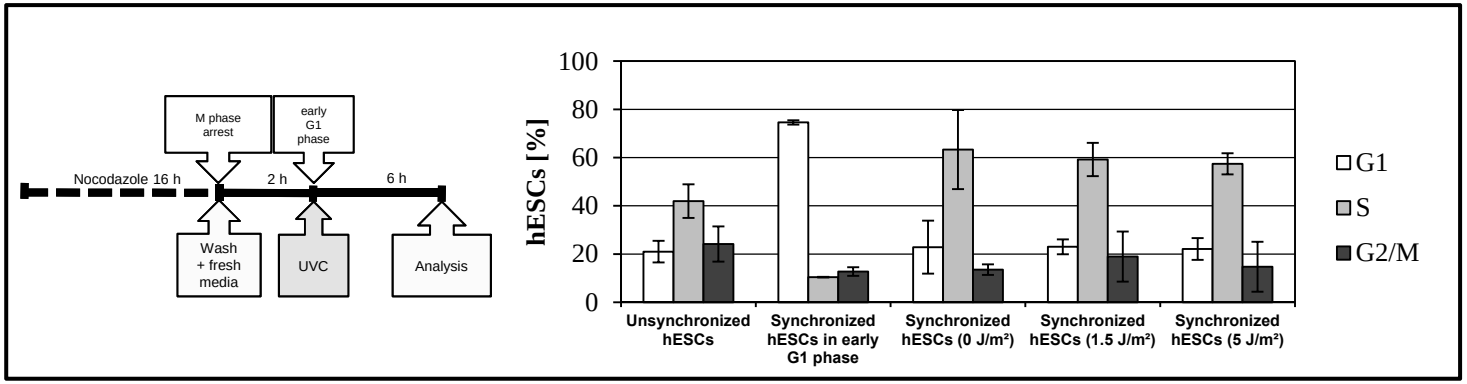
Dolezalova et al., Stem Cells, 2011

Chk1 and Chk2 mediate response to UVC in hESC.









Barta et al., Stem Cells, 2011

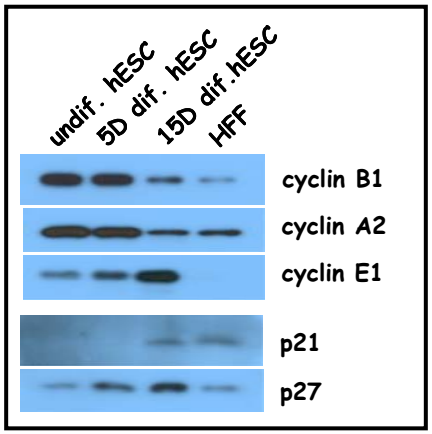
Conclusions 1

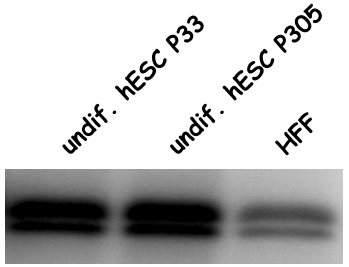
hESCs have limited capability to execute cell cycle chcekpoints upon damage to their DNA.

microRNAs that are responsible for inability to fruitfully activate p53-p21 DNA damage axis are among those that are specific for stem cell phenotype of hESCs.

At least in hESCs, microRNAs seem to provide an interconection among differentiation status, cell cycle prgression and DNA damage response.

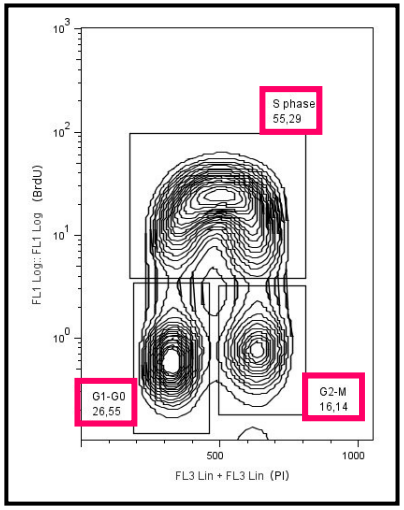
Cell cycle progression is unusual in hESCs.

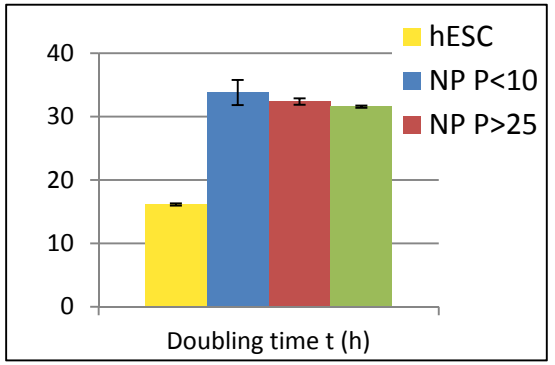




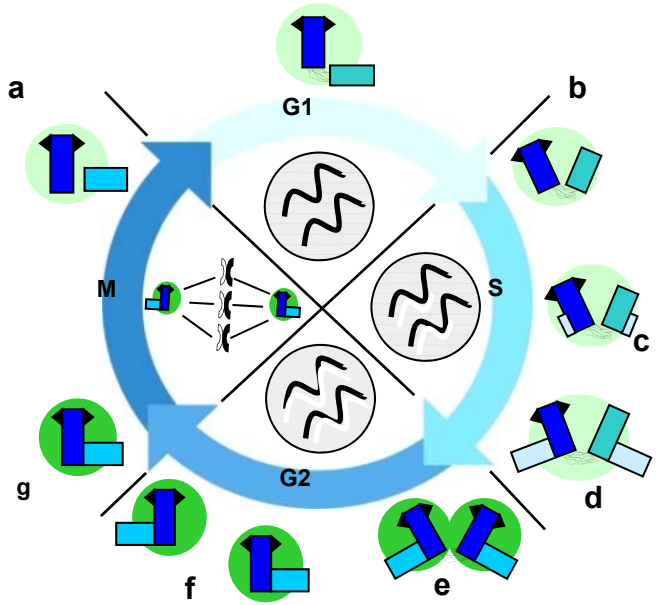
CDK2 kinase activity

High activities of CDK1 & CDK2



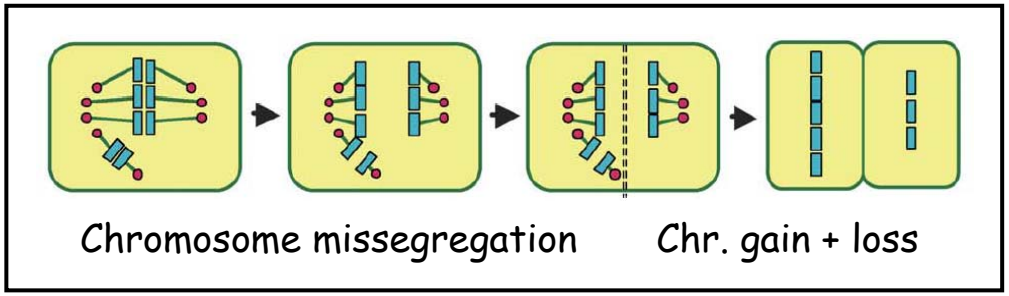


Centrosome cycle is driven by CDK2.

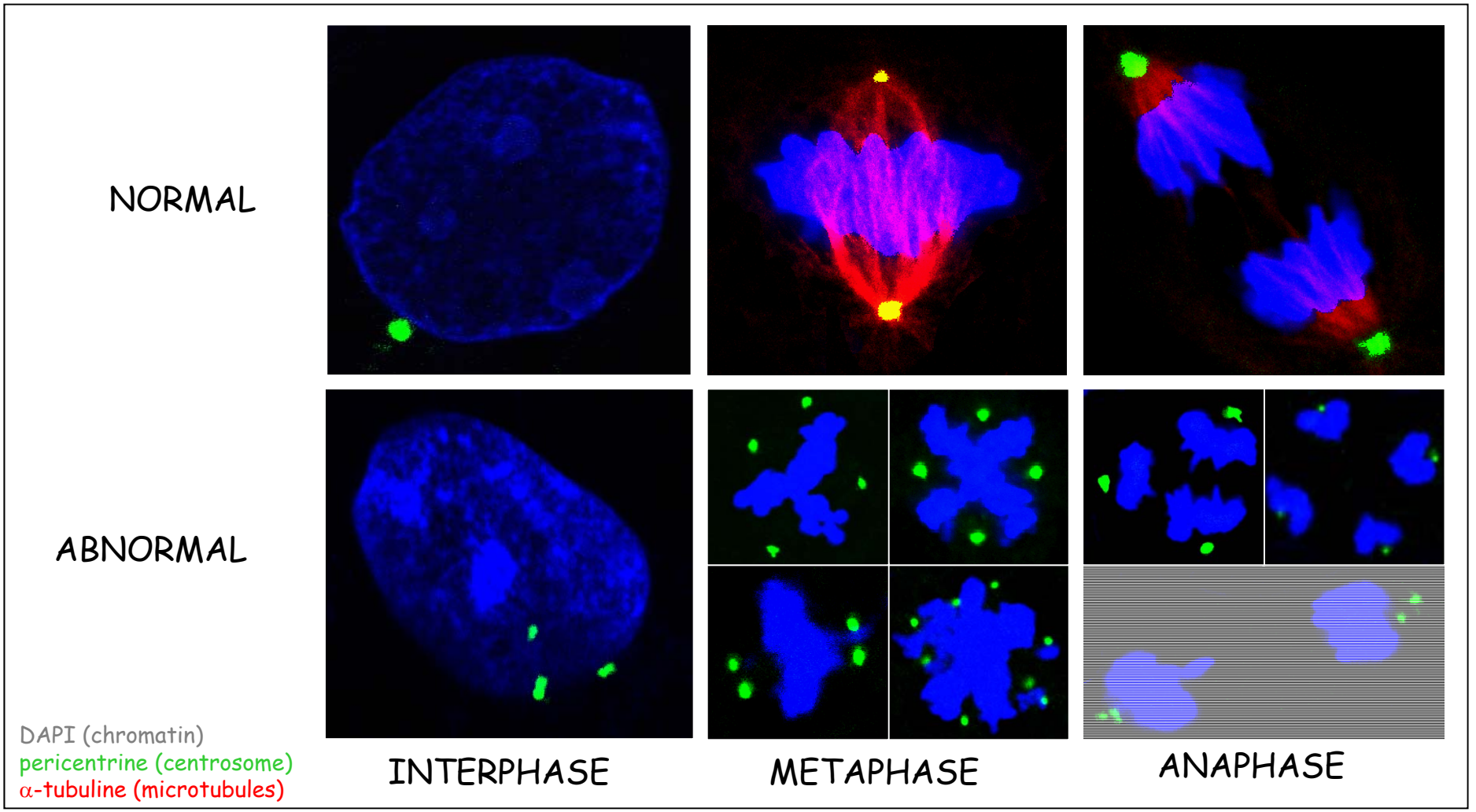


Duplication of centrosome is:

- driven by the activity of CDKs
- linked to cell polarity
- linked to cell anchorage
- linked to symmetric/asymmetric division
- linked to the activity of FGFR1
- checked by p53-dependent mechanism
- dependent on fuctional Rb protein



Cultured hESCs display centrosomal overamplification that produce abberant mitoses.



In hESC supernumerary centrosomes develop with very high frequency !

Undifferentiated hESC

cell line	passage number	mitoses multicentrosomal / total	multicentrosomal mitoses percentage
CCTL6	P26	18 / 88	20,45 %
CCTL8	P24	31 / 201	15,40 %
CCTL10	P14	61 / 260	23,46 %
CCTL12	P18	17 / 158	10,82 %
CCTL13	P18	10 / 68	14,70 %
CCTL14	P19	38 / 237	16,03 %
HS181	P25	18 / 108	16,60 %
HS420	P31	21 / 172	12,02 %
HS207	P27	10 / 61	14,75 %
HS306	P39	21 / 131	16,03 %
HS401	P23	15 / 146	10,27 %
HUES9	P27	57 / 544	10,47 %

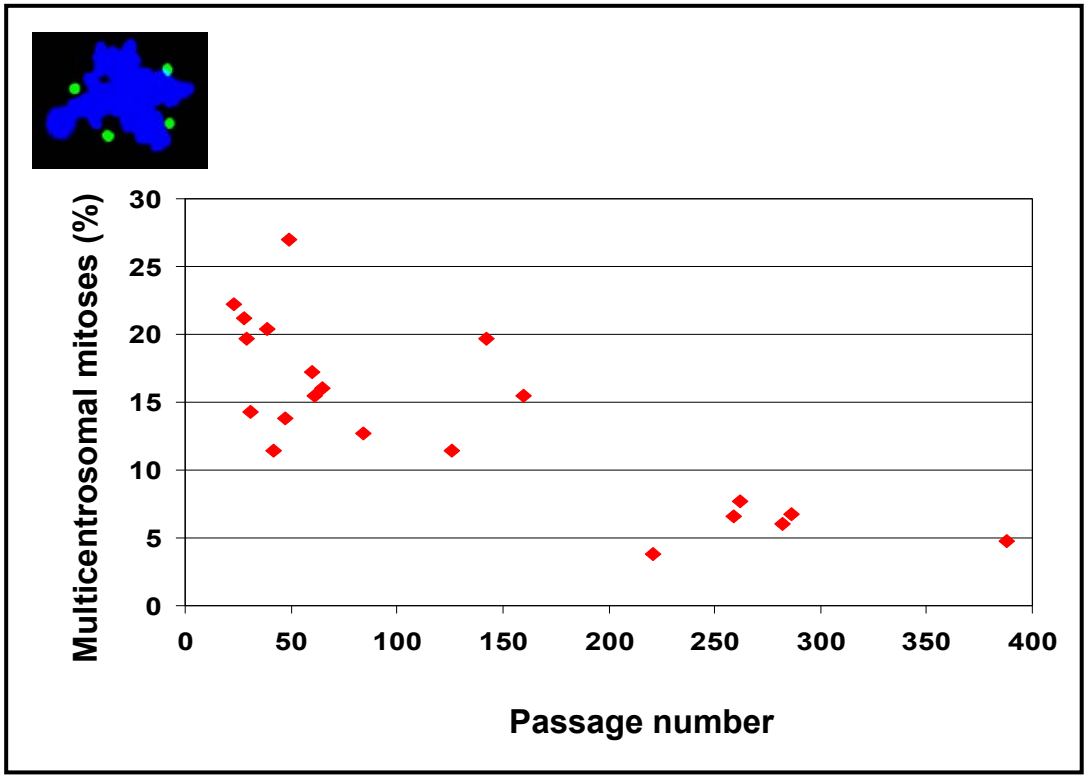
Brno

Stockholm

Boston

Holubcova et al., Stem Cells, 2010

Prolonged culture reduces the frequency of mitoses with supernumerary centrosomes.

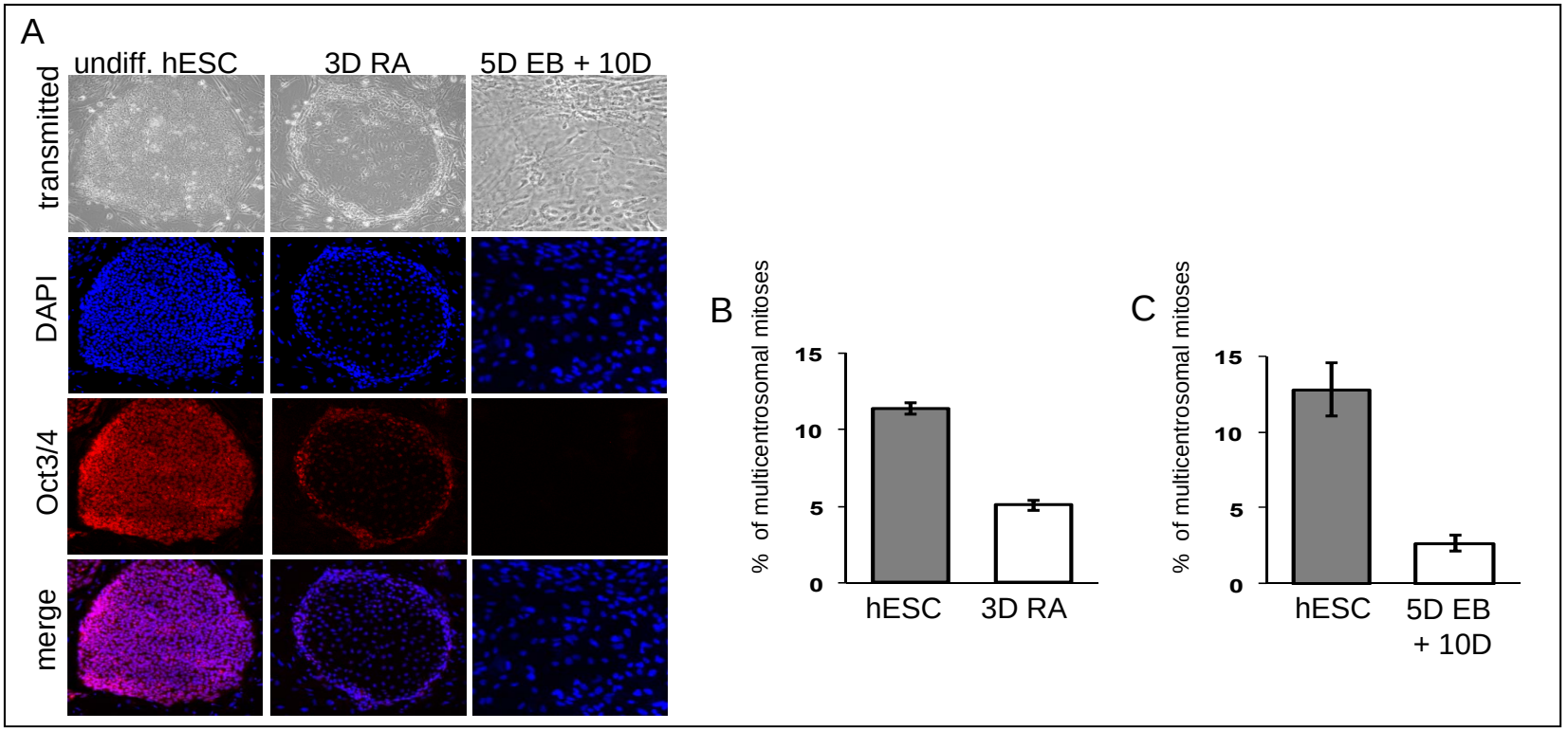


Holubcova et al., Stem Cells, 2010

Differentiated cells

cell line	mitoses multicentrosomal / total	multicentrosomal mitoses percentage
human foreskin fibroblasts (hFF) SCRC 1041	5 / 245	2,04 %
hESC derived fibroblast-like cells	1 / 37	2,70 %
β3Tu⁺/Pax6⁺ hESC-derived cells	5 / 106	4,71 %

Supernumerary centrosomes develop only in pristine hESC.



Holubcova et al., Stem Cells, 2010

In mESC the frequency of multicentrosomal mitoses is low !

cell line	passage number	mitoses multicentrosomal / total	multicentrosomal mitoses percentage
B10/CBA_11.1	P8	5 / 120	4,17 %
B10/CBA_11.2	P5	3 / 122	2,45 %
B10/CBA_11.3	P8	3 / 96	3,13 %
B10/CBA_11.4	P5	0 / 104	0,00 %
B10/CBA_11.5	P7	1 / 111	0,90 %
B10/CBA_11.6	P7	0 / 47	0,00 %
B10/CBA_11.7	P3	1 / 125	0,80 %
B10/CBA_11.8	P4	3 / 109	2,75 %

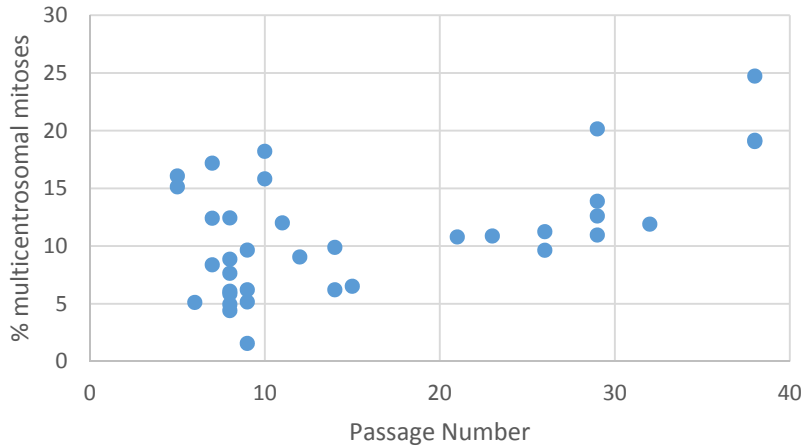
unpublished

In hiPSC the frequency of multicentrosomal mitoses varies depending on cell line !

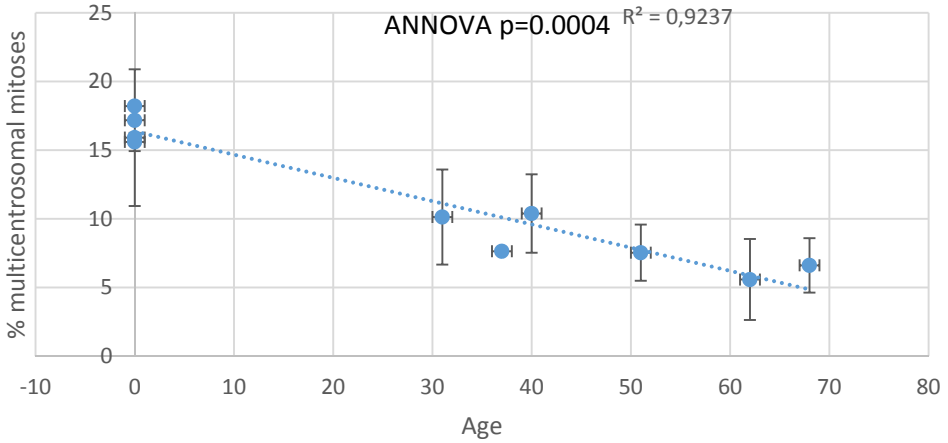
	Somatic cells		hiPSC		
fibroblast source	multicentrosomal / total mitoses	multicentrosomal mitoses percentage	clone ID (passage number)	multicentrosomal / total mitoses	multicentrosomal mitoses percentage
Human foreskin fibroblasts	0/96	0,0%	HFF_L1 (P20)	10 / 110	**9,09%**
			HFF_L2 (P20)	5 / 125	**4,0%**
Normal human dermal fibroblasts (Lonza)	6/60	10,0%	NHDF (P26+7)	14 / 202	**6,9%**
Adult dermal human fibroblasts	2 / 267	0,74%	AHDF_#1 (P36	25/249	**10,07%**
			AHDF_#4 (P35)	29/217	**13,36%**
Ligase IV mutated (patient derived)	0 / 60	0,0%	FO7/614 (P18+10)	5 / 110	**4,5%**
	4 / 111	3,6%	FO7/614_shRNAp53 (P20+11)	29 / 174	**16,6%**
	0 / 52	0,0%	GM16088 (P19+9)	1 / 77	**1,29%**
	0 / 56	0,0%	GM17523 (P18+6)	20 / 160	**12,5%**

unpublished

Percentage of multicentrosomal mitoses related to passage number



Percentage of multicentrosomal mitoses related to the age of donor



unpublished

Supernumerary centrosomes are structurally normal.

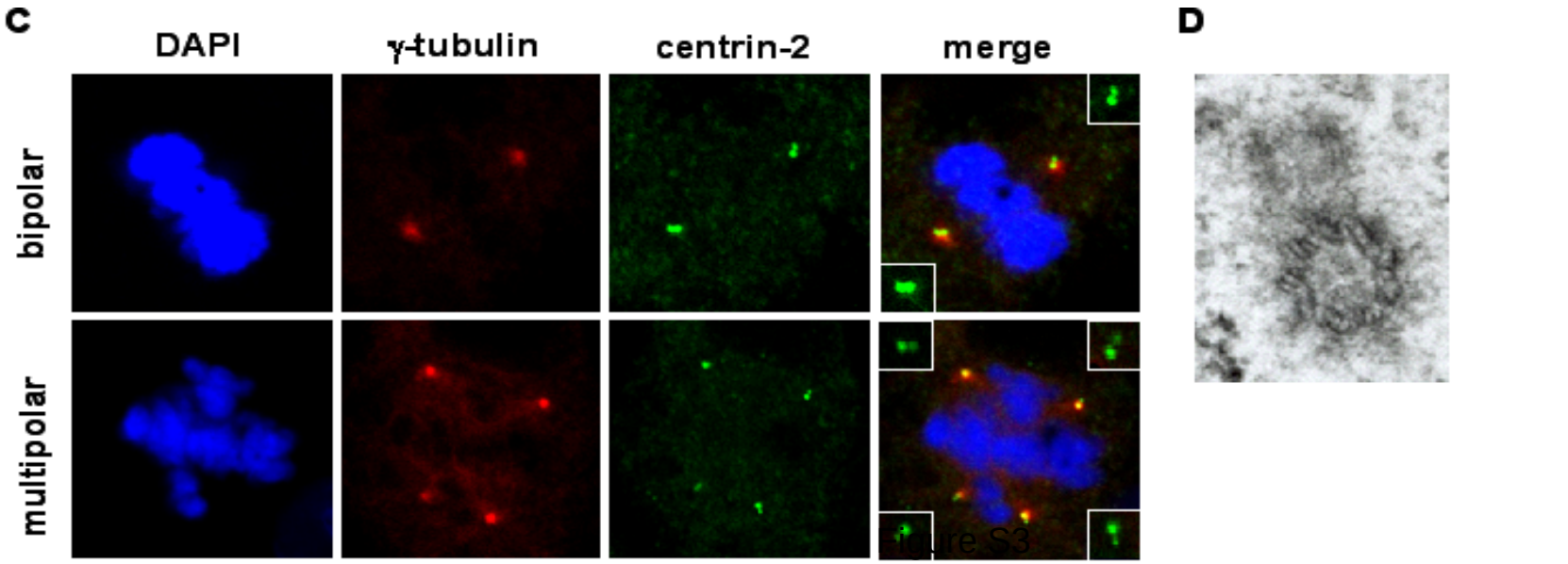
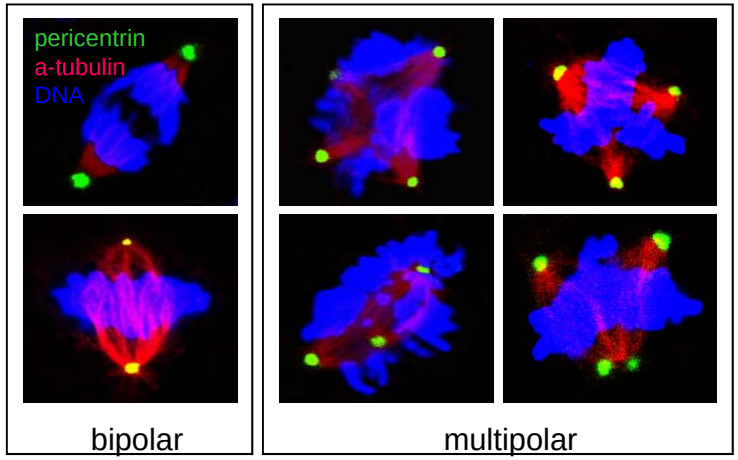
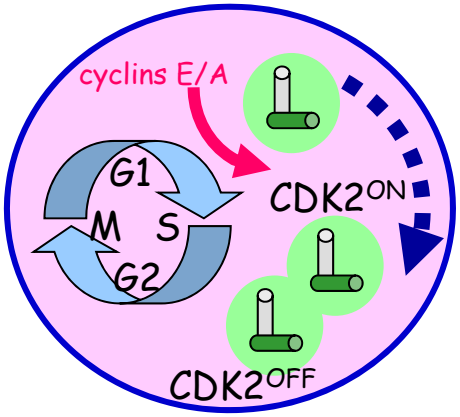


Figure S3

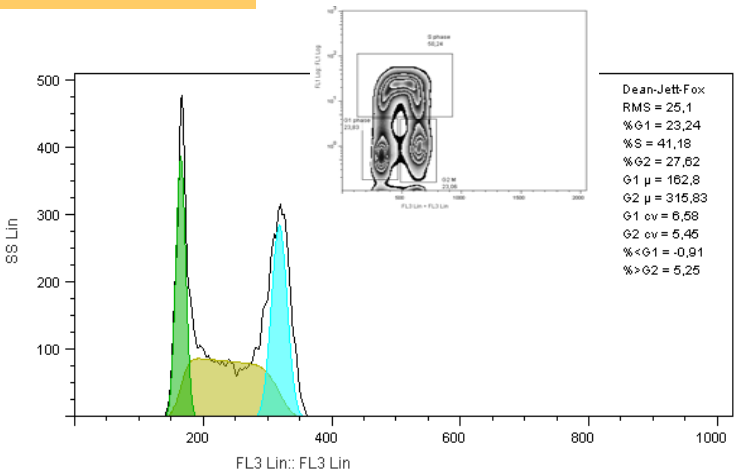


Holubcova et al., Stem Cells, 2010

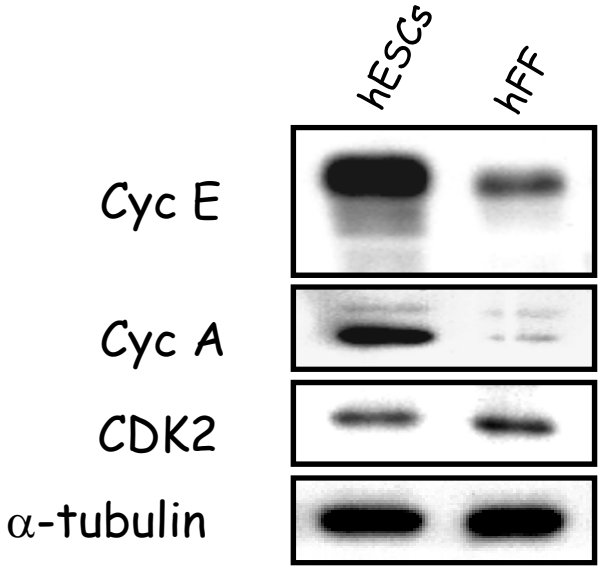
CDK2 – driver of centrosome duplication.



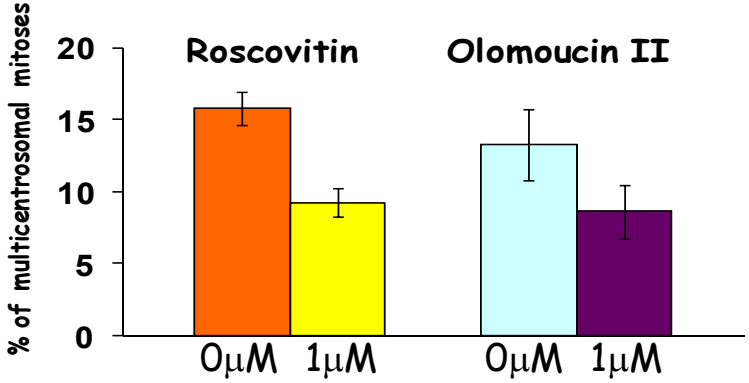
CELL CYCLE



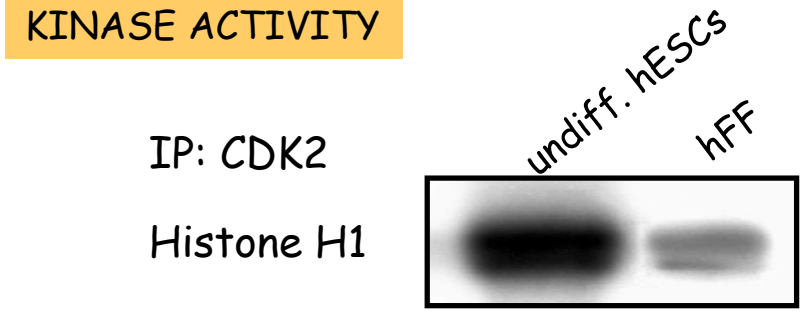
QUANTITIES



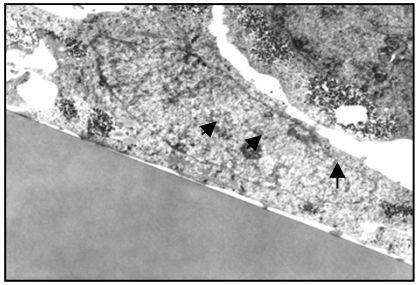
CHEMICAL INHIBITION

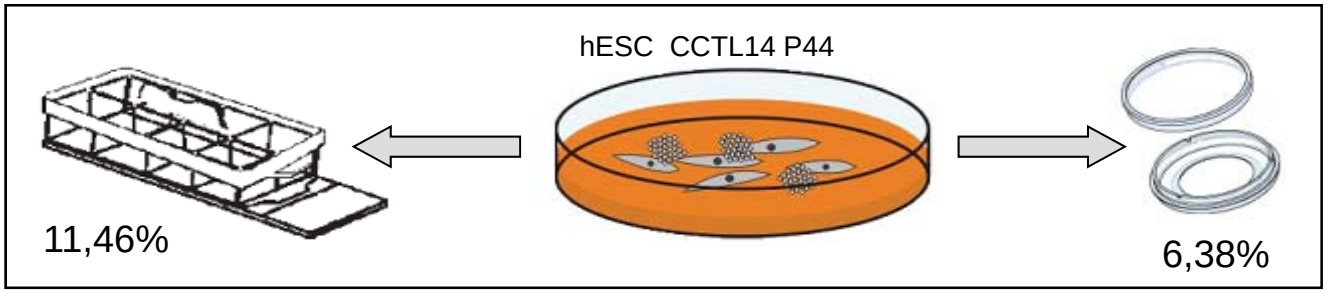


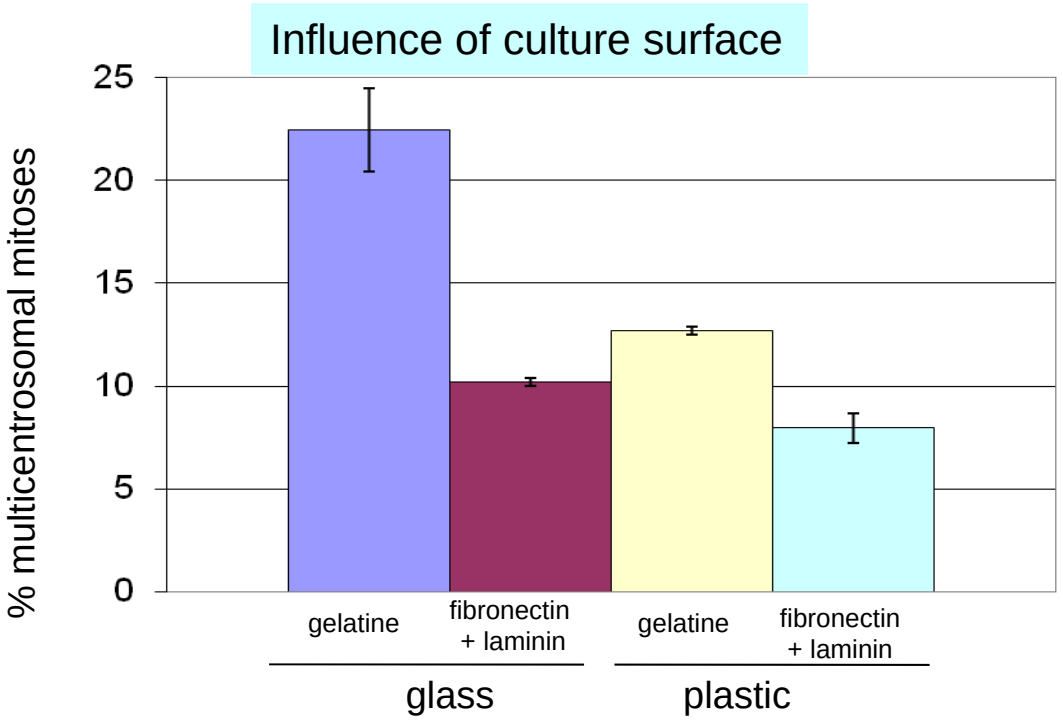
KINASE ACTIVITY

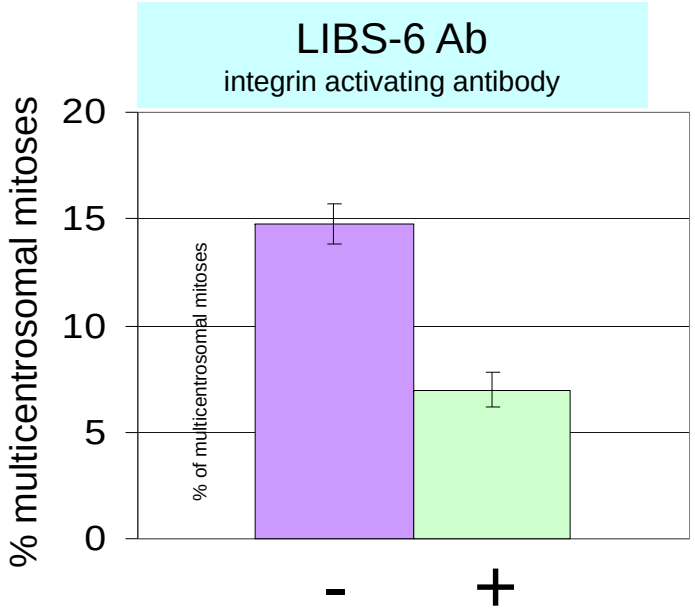


Quality of cell adhesion impacts on the frequency of supernumerary centrosomes.



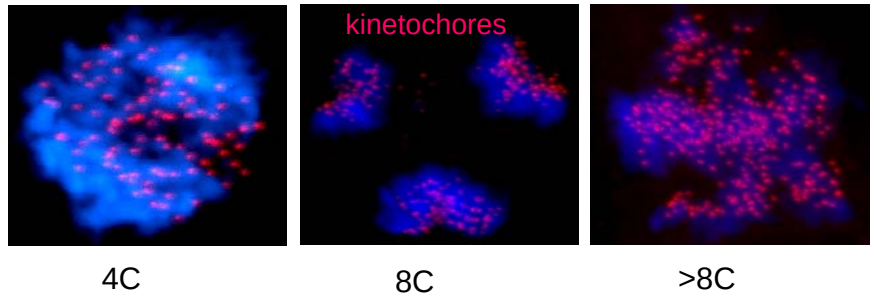






Holubcova et al., Stem Cells, 2010

Both endoreduplication and mitotic failure contribute to overamplification of centrosomes in hESC.



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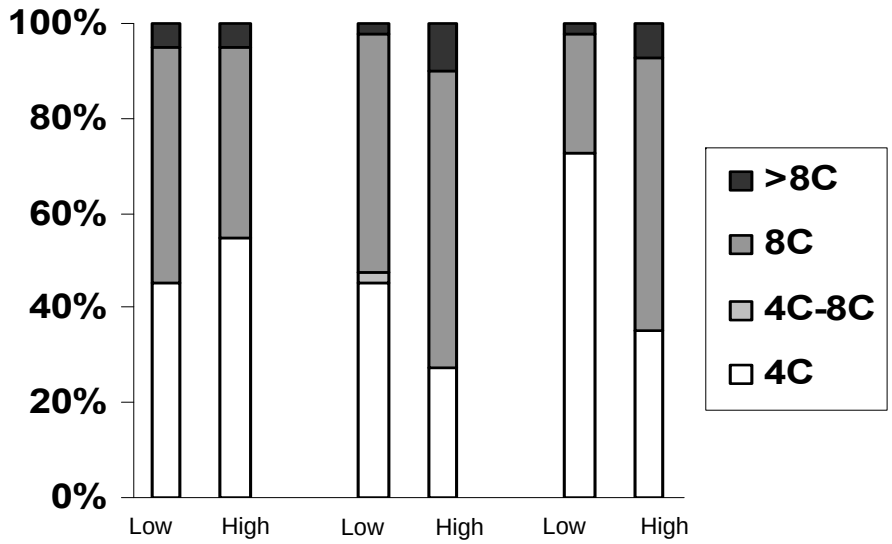


CCTL10 CCTL12 CCTL14

Holubcova et al., Stem Cells, 2010

Conclusions 2

Centrosomal overamplification is typical for undifferentiated state and early passage hESC and to some extent also to hiPSC.

During prolonged culture hESC seem to acquire „mutations“ that provide growth advantage by suppressing centrosomal abnormalities, which are antagonistic to cell viability.

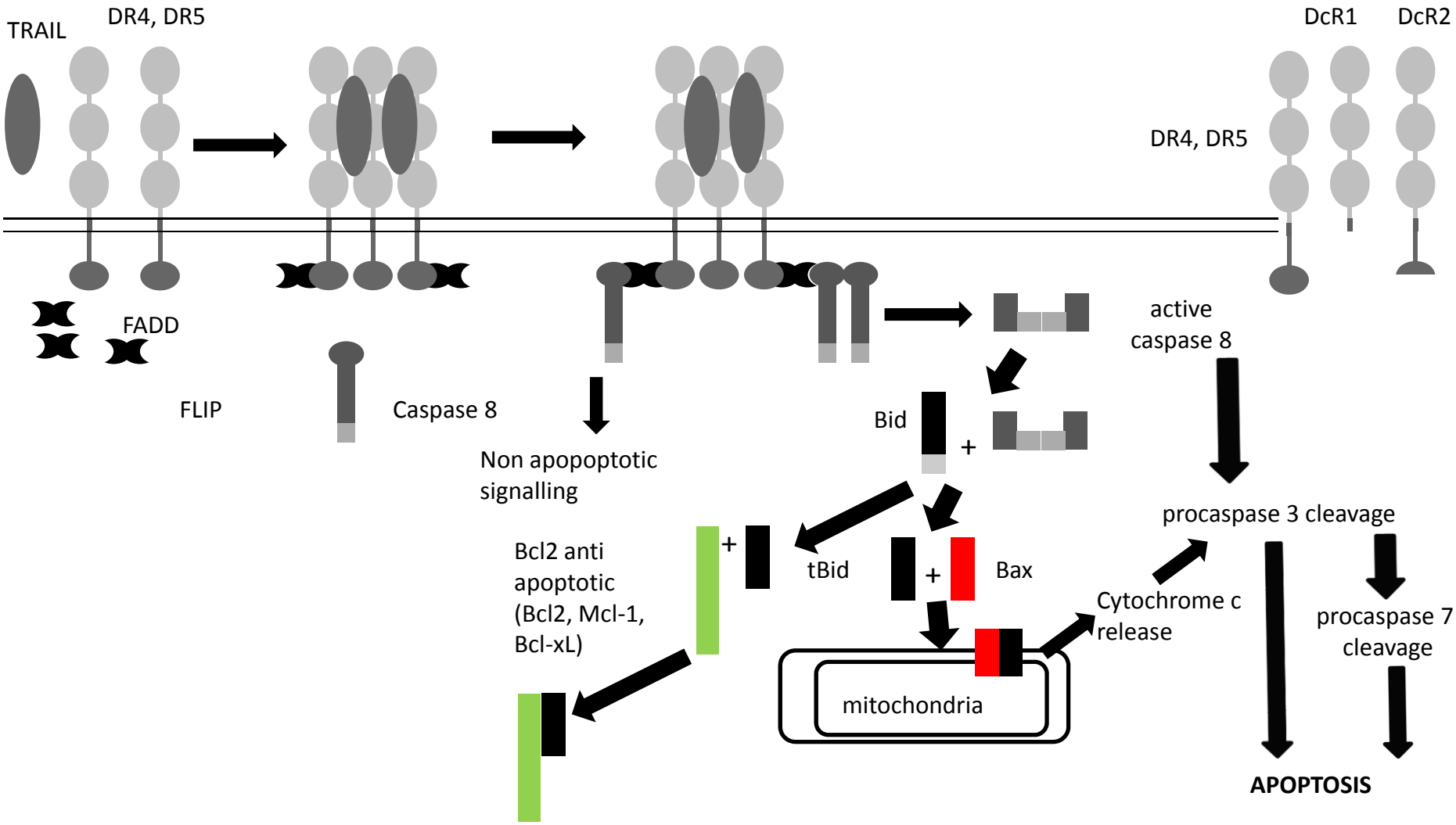
Functional supernumerary centrosomes in hESC generate conditions that lead to formation of multipolar spindles, which may produce suboptimal chromosomal segregation and aneuploidy.

They are ways how to influence „metabolism“ of stem cells to lower the possible risks associated with stem cell specific behaviors.

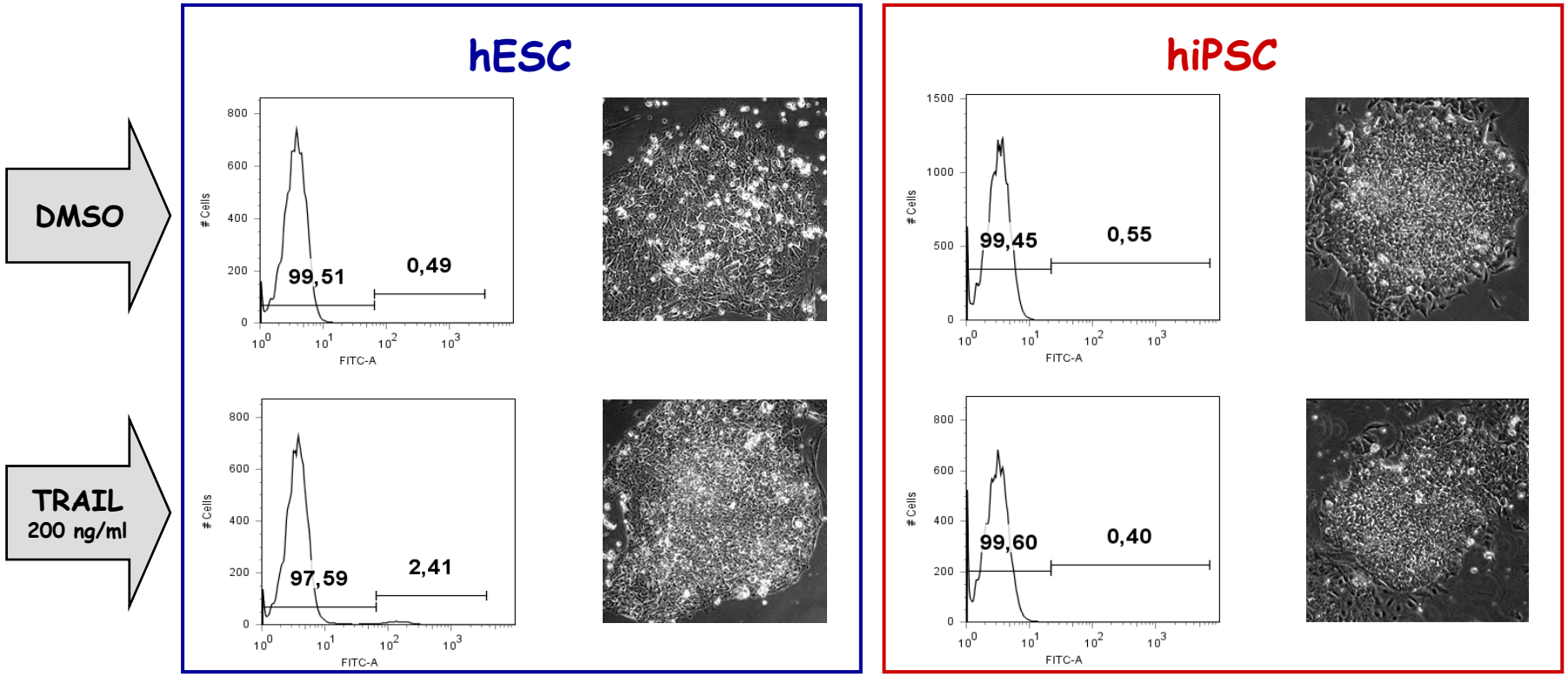
Unravelling and understanding these stem cell specific phenomena is instrumental for elimination of the risks.

Dr. Rao: Road-block „Limited expertise in scale-up manufacturing“

Functioning of extrinsic cell death pathway in hESC ?



hESC and hiPSC do not undergo apoptosis upon TRAIL induction.



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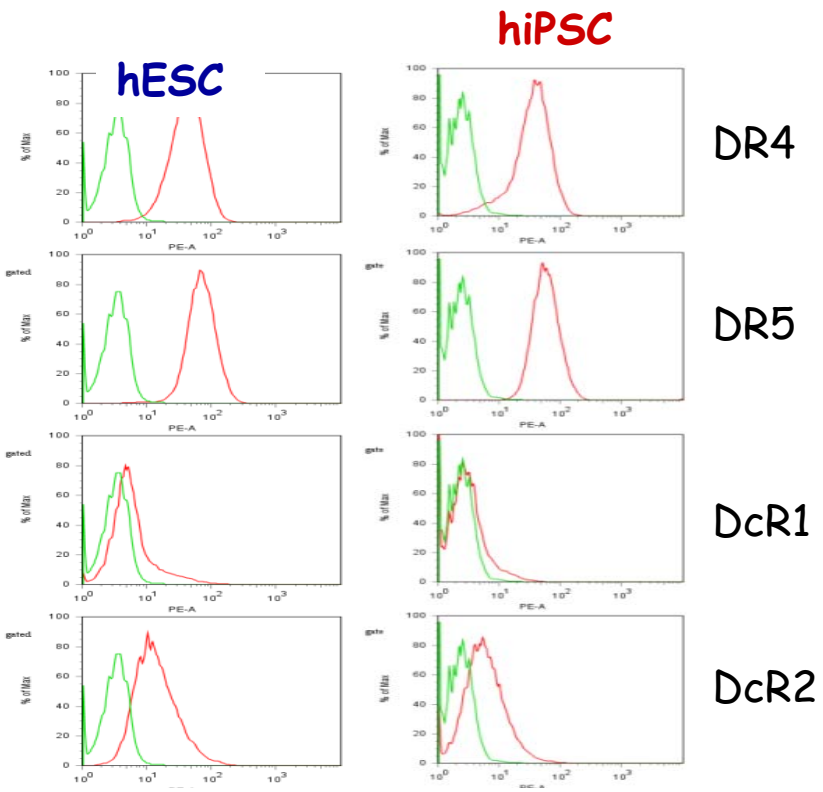
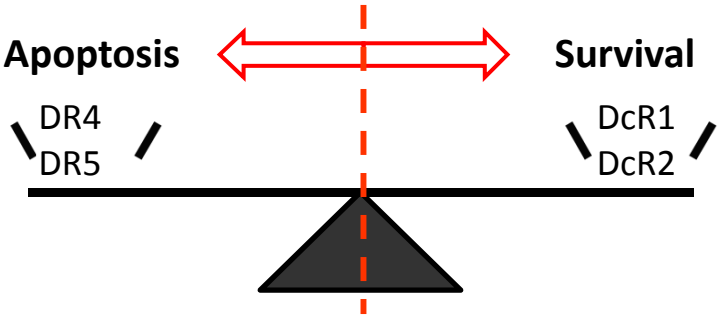
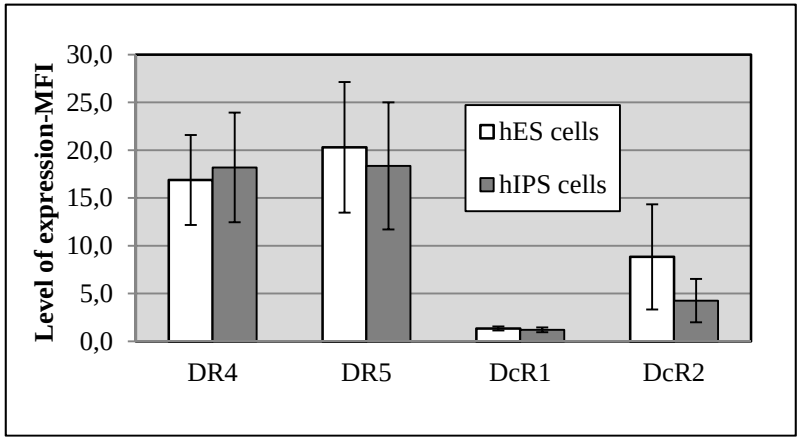
hESC and hiPSC express proapoptotic TRAIL receptors.







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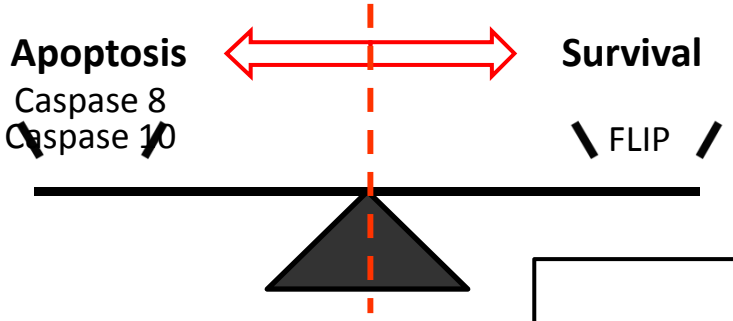
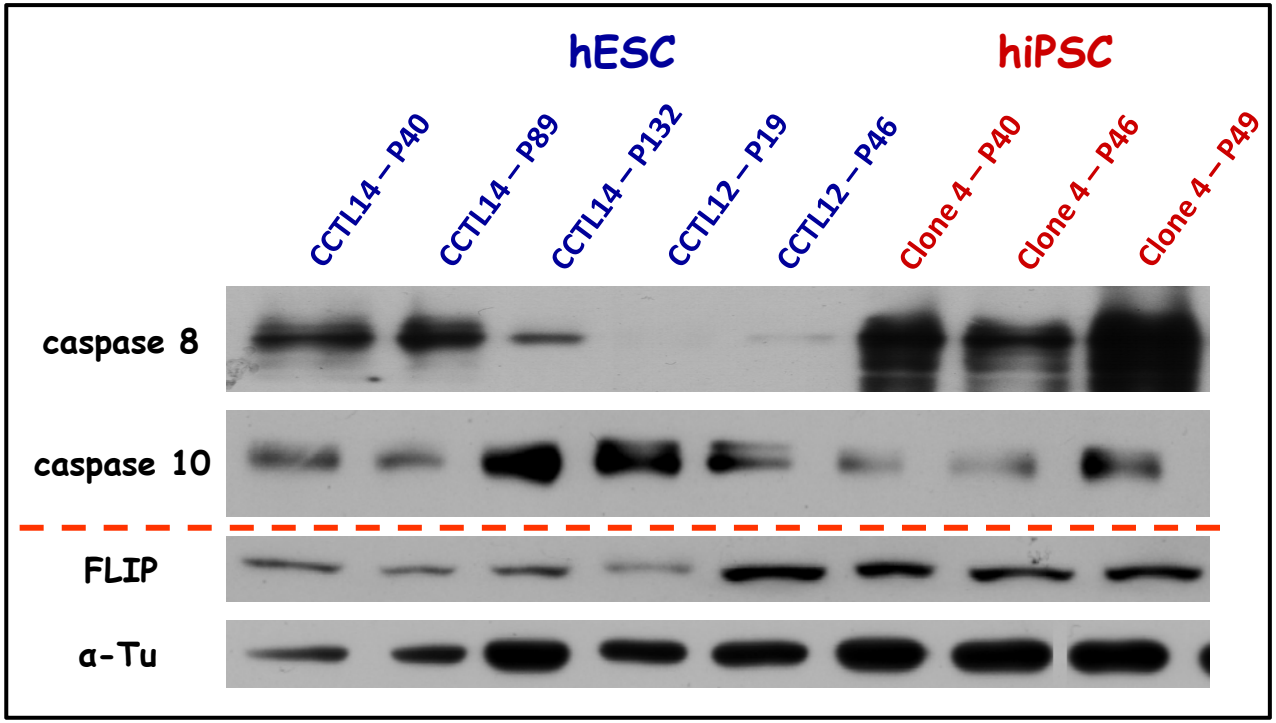
hESC and hiPSC possess components of the DISC.





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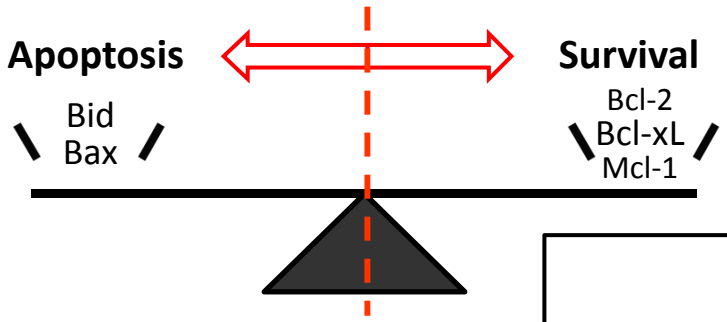
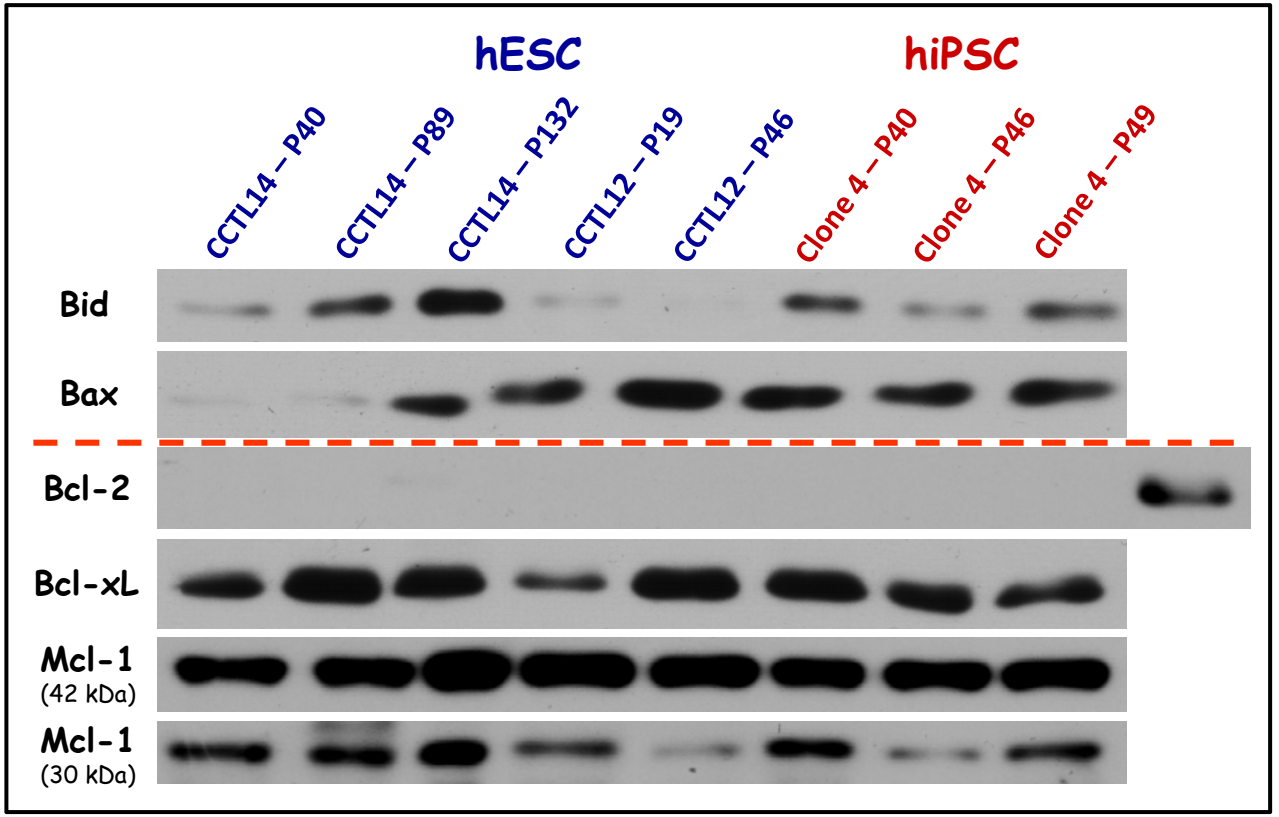
hESC and hiPSC possess members of Bcl family.





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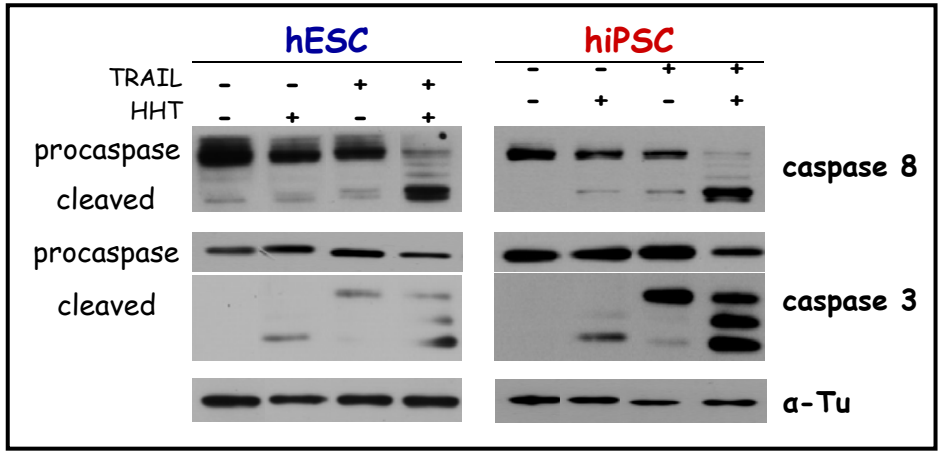
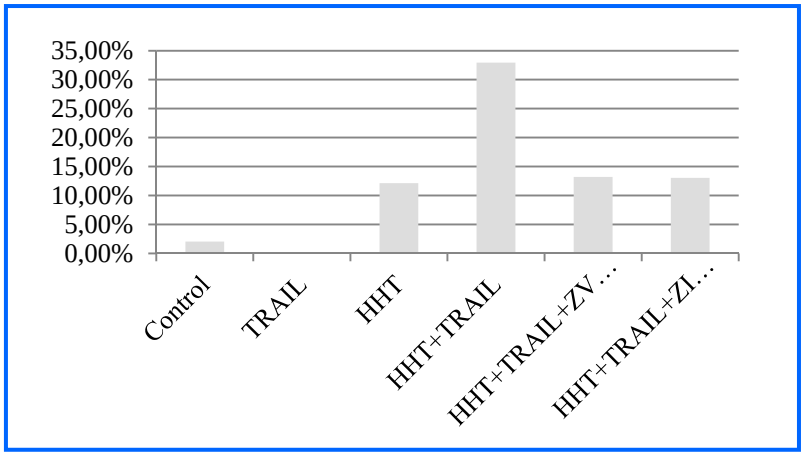
Homoharringtonine (HTT) sensitizes hESC TRAIL-induced apoptosis.



Caspase inhibition



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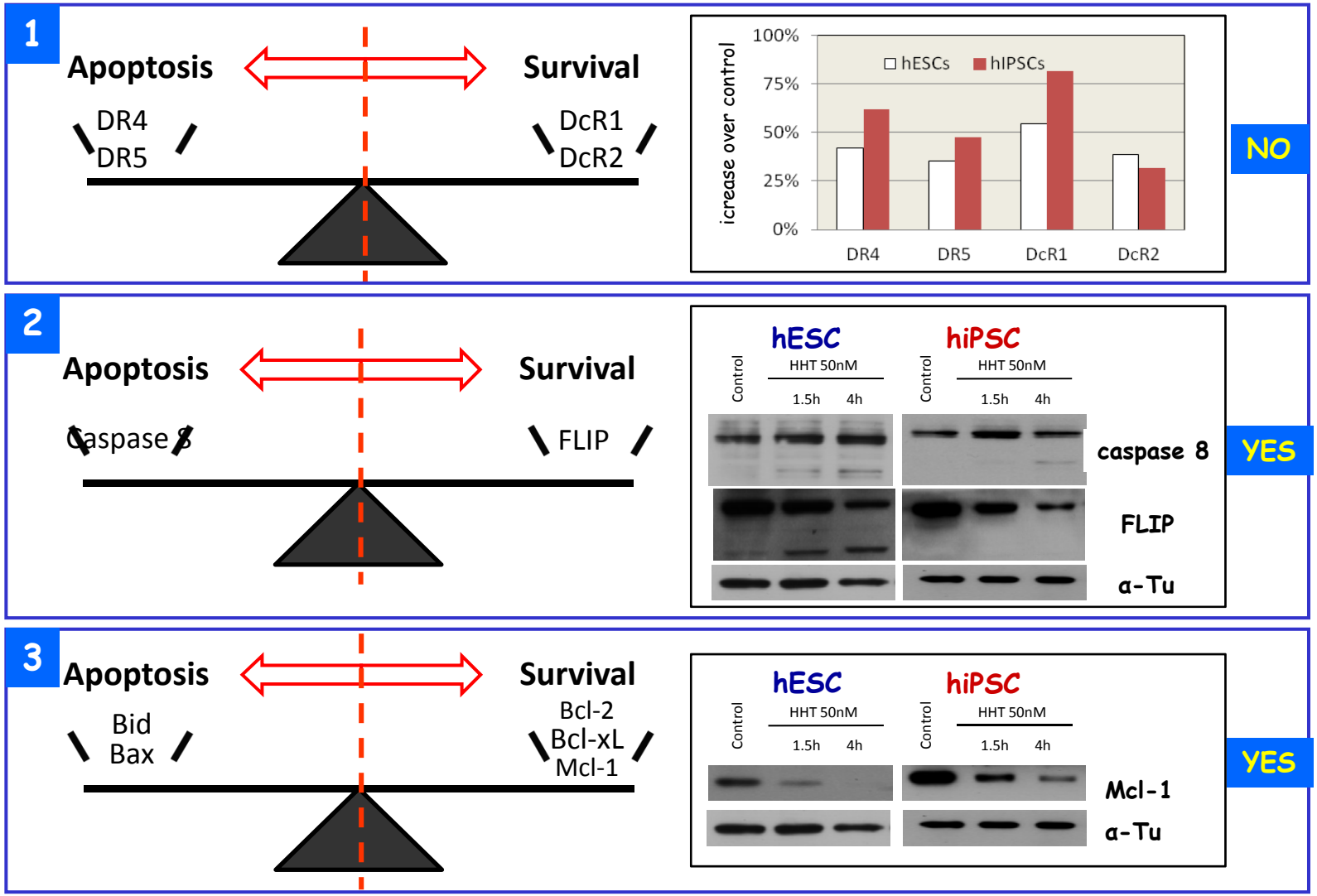
Mechanisms underlying HTT sensitisation



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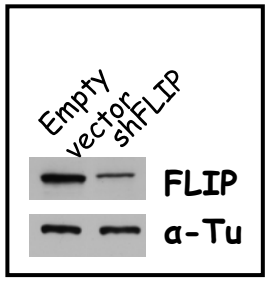
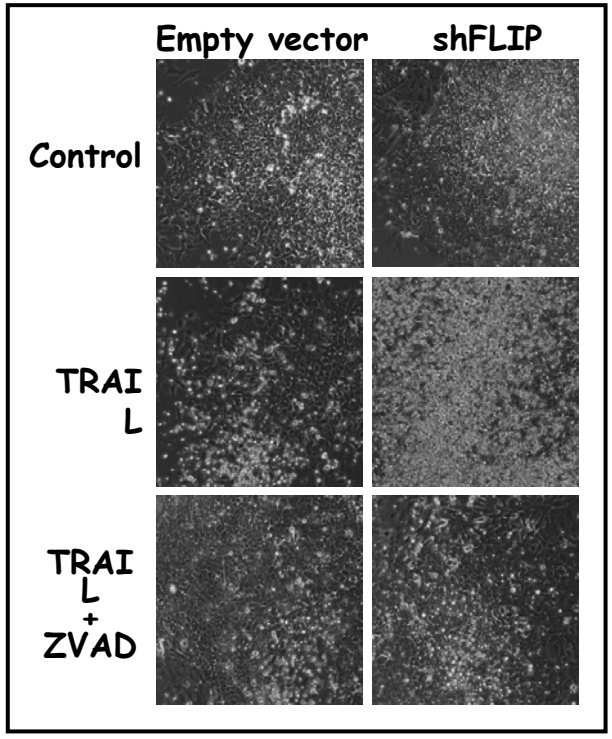
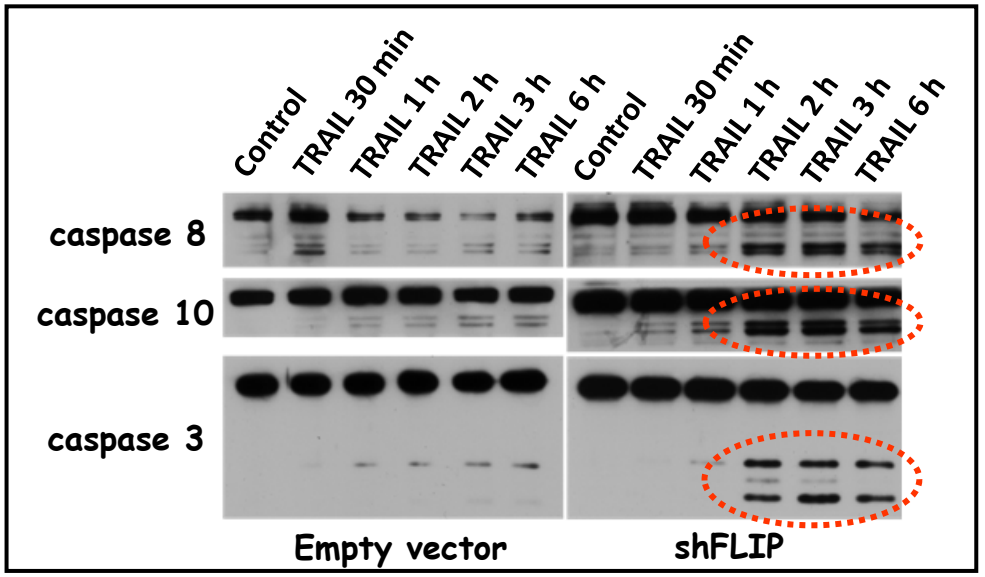
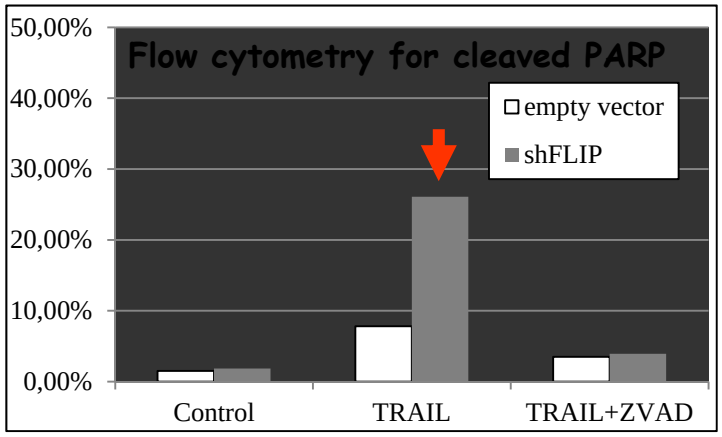
Down-regulation of FLIP predisposes hESC to TRAIL-induced apoptosis.









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Conclusions 3

Although they are molecularly equipped to receive and transmit TRAIL-delivered death signals, both hESC and hiPSC are inacapable of executing TRAIL-induced cell death.

hESC and hiPSC can be primed for TRAIL-induced cell death by chemical sensitisation, for example by inhibitor of proteosynthesis – Homoharringtonine.

Downstream regulators, such as FLIP and Mcl-1, and not TRAIL receptors, are responsible for TRAIL resistance of hESC and hiPSC.


Thank you for your attention !
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