Introduction to Pluripotent Stem Cells

Gastrointestinal block-Anatomy-Lecture 8

Editing file

MED438
KING SAUD UNIVERSITY

Anatomy team
med 438

Reviewed by Razan AlRabah & Ibrahim AlDakhil

Objectives

Color guide :
Only in boys slides in Green
Only in girls slides in Purple
important in Red
Notes in Grey

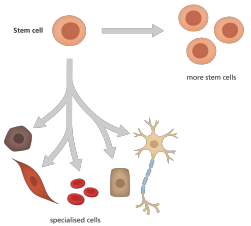
🍎 At the end of the lecture, students should be able to:

- Stem Cell : Definition & main function within the body.
- Where can we find Stem Cells (location).
- Classifications of stem cells:
  - Embryonic Stem Cell
  - Adult stem cells (Tissue Specific Stem Cell)
  - Induced Pluripotent Stem Cell (iPS) cells
- Different approaches for isolation of pluripotent stem cells.
- The Promise of Stem Cell Technology.

Stem Cells (Introduction)

A cell that has the ability:

1. To continuously divide and give rise to new copies of itself (Unlimited self-renew).
2. Differentiate into various kinds of cells/tissues:
   a. Endoderm (Hepatocytes)
   b. Mesoderm (Cardiac myotubes)
   c. Ectoderm (Neurons)



Unique Characte-ristics:

- Unlimited self renew (Regeneration)[1]
- Differentiation [2] (eg. beating cells of the heart muscles)

-Internal signals (specific genes)
-External signals (GF, cytokines)

Main function within the body:

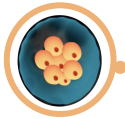
★ Continuous Repair of defective cell types and regeneration of tissues.

For tissue homeostasis or health to be maintained there has to be a balance between these two characteristics.
If there is imbalance:
1. Too much regeneration and unlimited dividing will result in **cancer**
2. Too much differentiation without enough regeneration will lead to **aging and degeneration** (die)

Classification Of Stem Cells

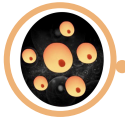
5 MINUTES

A. Potency[1] based



1. Totipotent[2] (Total) (morula)

2. Pluripotent[3] (plural) (blastocyst)



3. Multipotent (multiple)

4. Oligopotent

5. Unipotent

terminal cell

6. Nullpotent

B. Source based

1. Embryonic Stem Cells (ESC):

- **Include:** Totipotent and Pluripotent.
- **Sources:** IVF(in vitro fertilisation) embryos, Aborted embryos, cloned embryos.
- **Advantage:** Pluripotent & large number can be harvested.
- **Disadvantage:** May cause immune rejection & Ethical concerns.

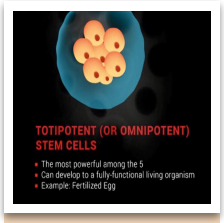
2. Adult Stem Cells (ASC)

- Multipotent, Oligopotent and Unipotent.
- **Sources:** Bone Marrow, Placental Cord & Mesenchymal Stem cells. (also fat tissue)
- **Advantage:** No immune rejection & No Ethical concerns.
- **Disadvantage:** Multipotent & Limited numbers and more difficult to isolate.
- **Found:** in specific mature body tissues as well as the umbilical cord and placenta after birth.
- They also can be isolated of developing embryos' different tissues.

3. Induced pluripotent stem cells (IPSCs)

1. The ability to divide.
2. Totipotent: from fertilization implantation .
3. Pluripotent: it is the inner cell mass after fertilization.

Classification cont. (potency based):



Totipotent (Total) (morula)

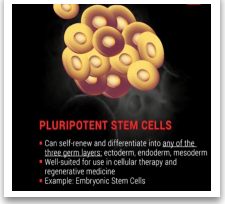
- Descendants of totipotent cells and differentiate into: cells of 3 germ layers (ectoderm, mesoderm, endoderm).

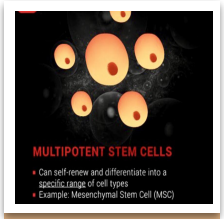
1

- 1-3 days, differentiate into (intra) embryonic and extraembryonic (like placenta, amniotic fluid and umbilical cord) cell types.
- this type can differentiate into anything.

2

Pluripotent (plural) (blastocyst)





Multipotent (multiple)

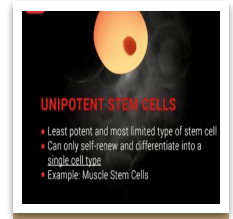
3

- Produce cells of closely related of cells (e.g. hematopoietic from bone marrow) family stem cells.
- Examples: Mesenchymal stem cells, Hematopoietic stem cells.

4

- Differentiate into ONLY a few cells, such as: lymphoid or myeloid stem cells.

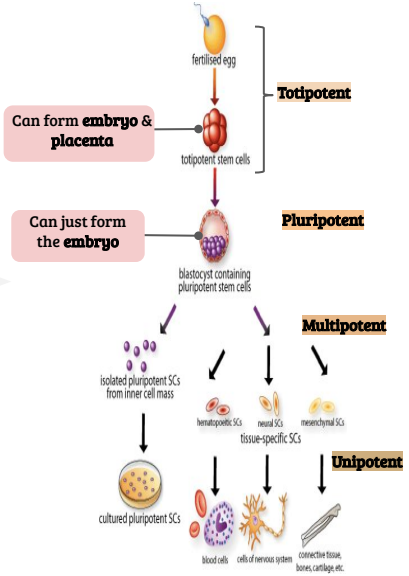
Oligopotent



Unipotent

5

- Produce ONLY one cell type (e.g. muscle stem cells).



Induced Pluripotent Stem Cells (IPSCs):

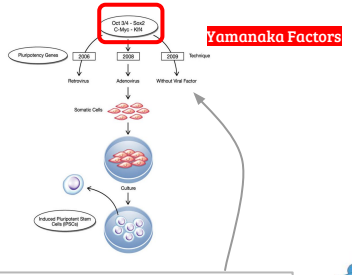
5 MINUTES

01 In late 2006 the group of Takahashi and Yamanaka reported the stimulation of cells of adult and embryonic origin to pluripotent stem cells called induced pluripotent stem (iPS) cells.

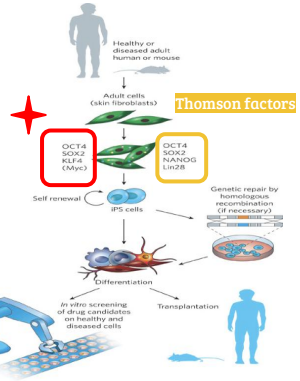
02 The method was described by Yamanaka and takahashi in which the skin cells of laboratory mice were genetically manipulated and returned back to their embryonic state.

03 **iPS** are somatic cells that have been reprogrammed to a pluripotent state (embryonic stem cell like state).

04 Several difficulties are to be overcome before iPS cells can be considered as a potential patient-specific cell therapy. It will be crucial to characterize the development potential of human iPS cell line in the future.



inject the factors to a virus specifically fiu virus because it can affect any cells

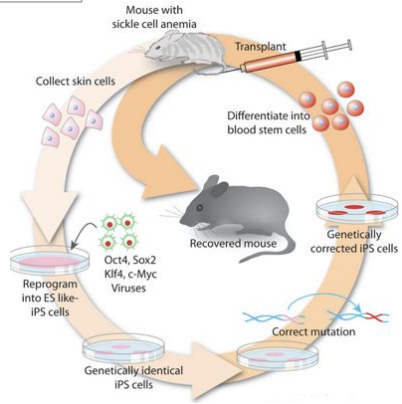


2nd: The cells were differentiated into hematopoietic cells.

01 02 03

1st: Skin cells were taken from the tail tip of a sickle-cell model mouse.

3rd: The produced cells were transfused back into the sick mouse.

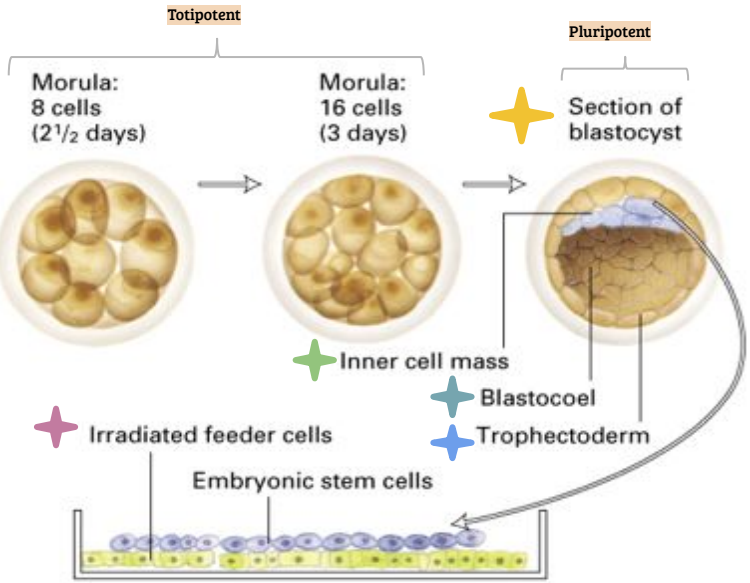


Males doctor said no need to know the method, just read it :)

Embryonic Stem Cells (ESC):

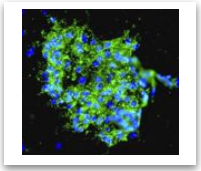
Generation of (ESC)

1. Embryonic human stem cells were first isolated in 1995 by dr. James thomson.
2. Derived from **4-5 day** old embryo (**blastocyst**): 1- **Trophoblast**, 2- **Blastocoel**, 3- **Inner Cell Mass** (ICS).
3. Isolate and transfer of **ICS** into culture dish in culture media Culture at 37 C° and 5% CO_2
4. Inner surface of culture dish is coated with inactivated **MEFs** (Mouse Embryonic Fibroblasts) as a **feeder cells**: Provides sticky surface for attachment & Release nutrients.
5. Cells divide and spread over the dish.
6. ESCs are removed gently and plated into several different culture plates.

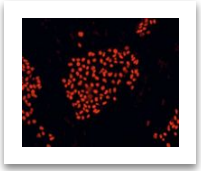


Human pluripotent stem cells (Embryonic Stem Cells) Characterization:

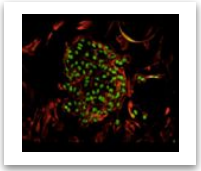
Specific markers for stem cell



SSEA-4

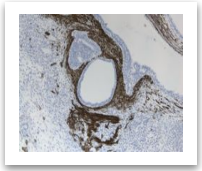


Sox-2



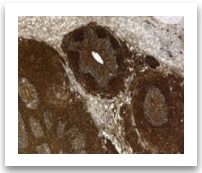
Oct-4

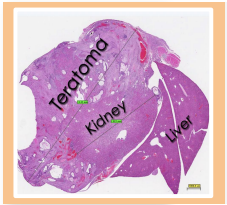
Endoderm:
Sox-17



Mesoderm:
Sox-9

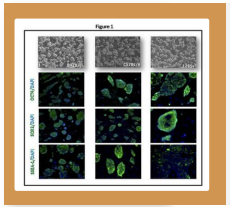
Ectoderm:
Beta- III tubulin





A large tumor mass measuring twice as the kidney is compressing it.

The teratoma was composed of mixed tissue patterns: skin with keratin, brain tissue, striated and smooth muscle, lymphoid tissue... (Which type of cells can cause teratoma ? Embryonic stem cells , Pluripotent cells



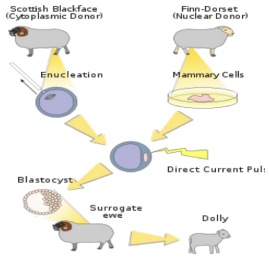
Teratoma Formation in Immunocompetent Mice After Syngeneic and Allogeneic Implantation of Germline Capable Mouse Embryonic Stem Cells, 2013.

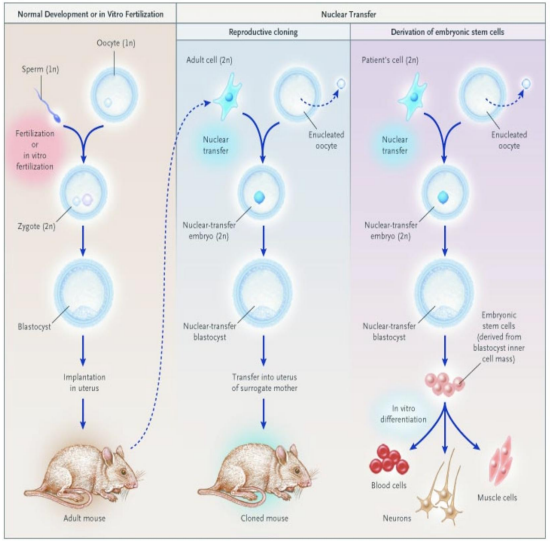
Somatic Cell Nuclear Transfer (SCNT) Cloning

01 Reproductive Cloning

- Reproductive cloning Involves creating an animal that is genetically identical to a donor animal **through somatic cell nuclear transfer.**
- Dolly is a sheep that was cloned from another sheep using the same method we discussed before.
  - An oocyte was deprived of its nucleus & a different nucleus was inserted and the blastocyst was reinserted into a surrogate mother.

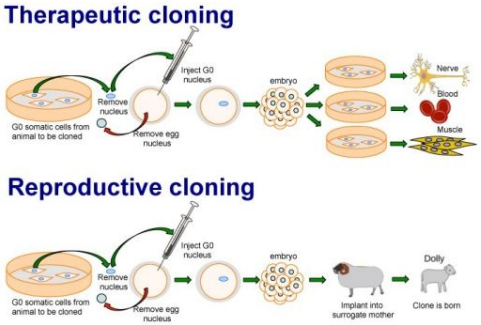
1. Firstly, They took a black face donor cell and removed the nucleus and DNA.
2. Secondly, from another donor, they took the DNA from mammary cells.
3. Then put the DNA in the anucleated cell.
4. Then stimulate with an electrical shock so the cell thinks it's getting fertilized and starts to develop.
5. Then they put it back In the blackface mother.
6. dolly came :)





02 Therapeutic Cloning

- Therapeutic cloning uses stem cells to correct diseases and other health problems that someone may encounter.
- Therapeutic cloning is not used to make full humans but rather is used for cloning the stem cells of the embryo.



The Challenges

With Embryonic Stem Cells (ESC)

1. Abnormalities in chromosome number and structure were found in some human ESC lines.
2. Stem cells need to be differentiated to the appropriate cell types before they can be used clinically.
3. Stem cell development or proliferation must be controlled once placed into patients (risk of teratoma formation).
4. The use of mouse “feeder” cells to grow ESC could result in problems due to xenotransplantation. (the feeder layer is supposed to be removed before we use the ESC but all isolation methods don't guarantee not having feeder layer in the sample. Now there are medias to grow without feeder layer)
5. Possibility of rejection of stem cell transplants as foreign tissues is very high.

&

The Promise

of Stem Cell Technology

1. Replacement of tissues/organs
2. Repair of defective cell types
3. Study cell differentiation
4. Toxicity testing.
5. Understanding prevention and treatment of birth defects.
6. Study of development and gene control.
7. Study of drugs therapeutic potential.

QUIZ

Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
D	B	B	C	C	B	A	C

Q1: Which of the following are pluripotent stem cells?

A. Cells has the potential to differentiate into any adult cell type forming an entire organism

B. Cells that has limited potential to form only multiple adult cell types

C. Cells that don't have the ability for self renewal

D. Cells has the Potential to form all differentiated cell types except placenta

Q2: Mesenchymal stem cells are examples of:

A. Pluripotent stem cells

B. Multipotent stem cells

C. Totipotent stem cells

D. Induced pluripotent stem cells (iPS cells)

Q3: The Blastocyst is formed of all of the following, EXCEPT:

A. Trophoblast

B. Morula

C. Inner Cell Mass

D. Blastocoel

Q4: Induced Pluripotent Stem Cell (iPS) cells are...

A. Cells have limited potential to form only multiple adult cell types

B. Cells are Potential to form all differentiated cell types

C. Somatic cells that have been reprogrammed to a pluripotent state

D. Cells are potential to differentiate into any adult cell type

Q5: Which one of the following is one of yamanaka factors:

A. Oct-2

B. Sox-17

C. Klf4

D. NANOG

Q6: Which one of the following cells could cause teratoma?

A. Oligopotent

B. Embryonated stem cells

C. Unipotent

D. Nullpotent

Q7: The marker of hPSCs that differentiate into ectoderm is:

A. Beta-III tubulin

B. Sox-17

C. Sox-9

D. Sox-2

Q8: The inner cells mass of embryo is which one of following?

A. Unipotent

B. Multipotent

C. Pluripotent

D. Totipotent

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med 438

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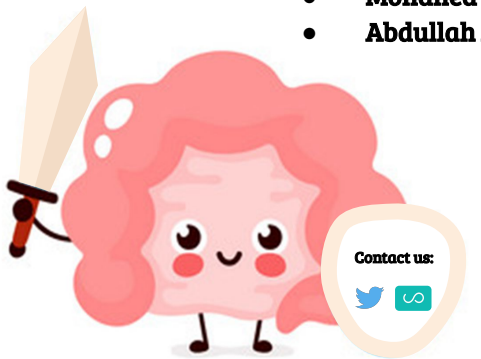

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