MED437
KING SAUD UNIVERSITY

ANATOMY
Team 437

Revised By
Hadeel Awartani
Abdullah AlOmar

Introduction to Pluripotent Stem Cells

Lecture (4)

- Important
- Doctors Notes
- Notes/Extra explanation

{وَمَنْ يَتَوَكَّلْ عَلَى اللَّهِ فَهُوَ حَسْبُهُ}

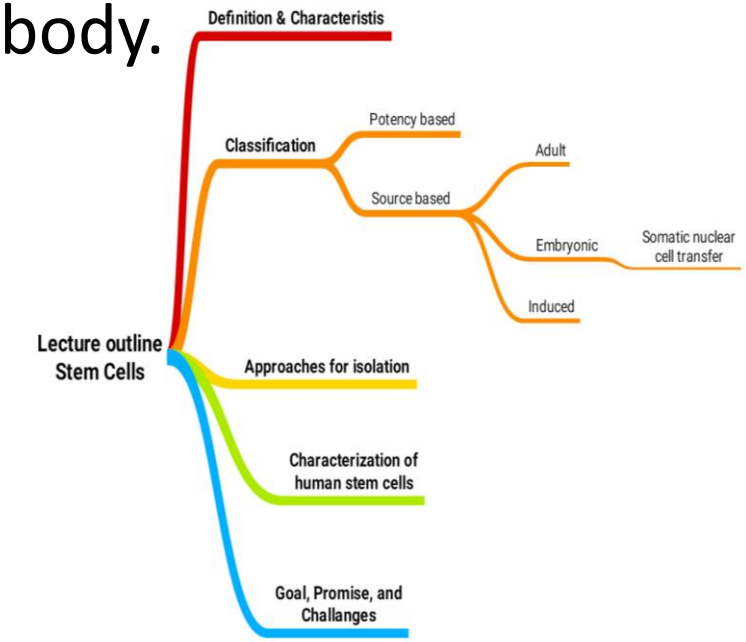
Please check our Editing File

هذا العمل مبني بشكل أساسي على عمل دفعة ٤٣٦ مع المراجعة
والتدقيق وإضافة الملاحظات ولا يغني عن المصدر الأساسي للمذاكرة

Objectives

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

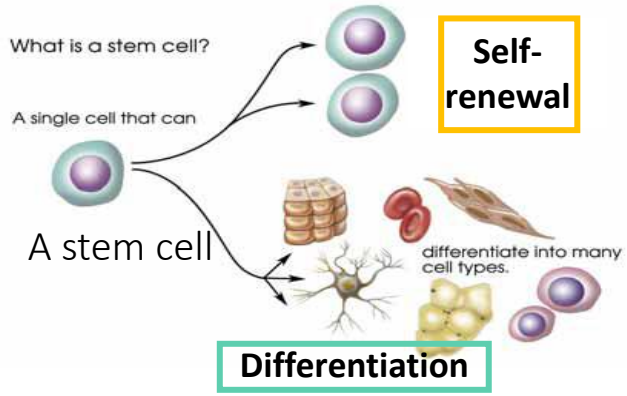
- ✓ **Stem Cell** : **Definition** & **main function** within the body.
- ✓ Where can we find **Stem Cells (location).**
- ✓ Classifications of stem cells:
  - **Embryonic Stem Cell**
  - **Adult stem cells (Tissue Specific Stem Cell)**
  - **Induced Pluripotent Stem Cell (iPS) cells**
- ✓ Different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



Stem cell:
-it is the first cell from fertilization
-undifferentiated
-it continues until death

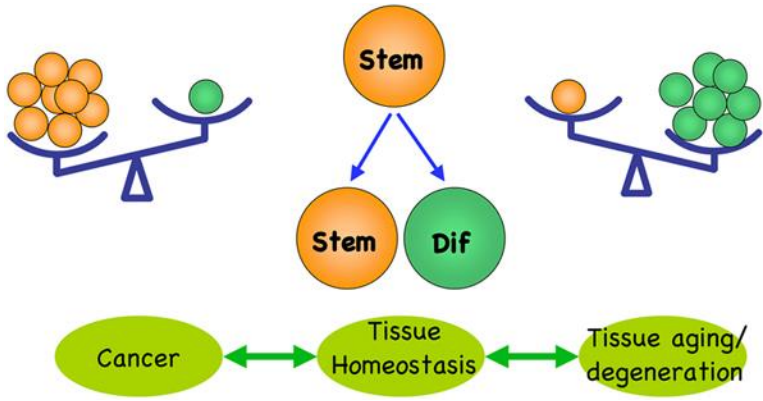
Stem Cells Introduction

- A cell that has the **ability**:
  - To continuously **divide** and **give rise to new copy of itself** (Unlimited self-renew)
  - Differentiate into various kinds of cells/tissues.
- **Unique Characteristics of Stem Cells:**
  - Differentiation (eg. beating cells of the heart muscles):
    - Internal signals (specific genes)
    - External signals (GF, cytokines) 2
- **Main function within the body:**
  - Continuous Repair of defective cell types and regeneration of tissues.



1- It divides to **give copies of itself** and this is the main idea behind **treating degenerative disease.**
2- They amplify the microenvironment **around cells**

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



IMPORTANT

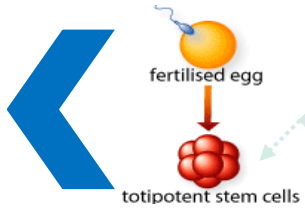
Classification of stem cells

I- (potency based) potency = the ability to divide

كل وحدة تعطي اللي تحتها

Totipotent (Total) (morula)

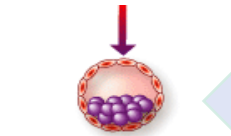
- 1-3 days, differentiate into (intra) **embryonic** and **extraembryonic** (like placenta, amniotic fluid and umbilical cord) cell types. (this type can differentiate into anything)



This cell can form the **Embryo & Placenta**

Pluripotent (plural) (blastocyst)

- Descendants of totipotent cells and differentiate into cells of **3 germ layers(ectoderm, mesoderm, endoderm) | Most important one that we use**



This cell can just form the **Embryo**

Multipotent (multiple)

- Produce cells of **closely related** of cells (e.g. hematopoietic from bone marrow) **family stem cells.**
- Used a lot in **clinical application** & **treated some diseases**

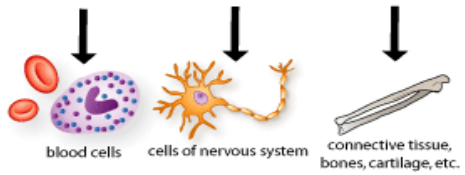
Oligopotent

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

Note: in the bone marrow, we have two types of stem cells:
1- hematopoietic (multipotent) → gives us blood cells (RBCs, WBCs)
2- mesenchymal stem cells (multipotent) → give us (bone, cartilage, skin, epithelial lining)

Unipotent

- Produce ONLY **one cell** type (e.g. muscle stem cells)
- Like the sperm, it differentiate before leaving the testis and like the cells in the ovaries



Classification of stem cells
II- (source based)

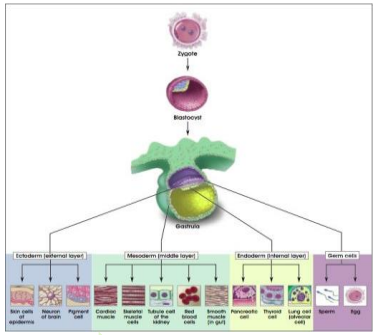
*Adult means any cell after day 14 when it becomes multipotent.
note that there is a difference between embryonic and fetus stem cells.
Fetus stem cells are considered adult stem cells

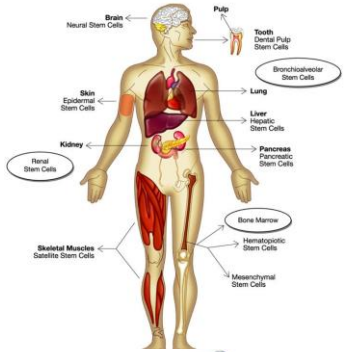
Totipotent: it is the fertilized egg until the 6th day where the implantation happens, after fertilization it divides into outer cell mass (extraembryonic) and inner cells mass (embryo).
The inner cell mass is the pluripotent stem cells.
Note: if you take one pluripotent stem cell, it can give you a full fetus (without extraembryonic tissue) and this is what happens in twins , but if you did the same thing with totipotent, this will give you a full fetus with the extraembryonic tissue

Embryonic means (totipotent and pluripotent)

Embryonic Stem Cells (ESC)

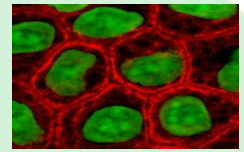
- Sources:
  IVF embryos, SCNT, Aborted embryos, cloned embryos
- **Pluripotent & large number can be harvested (Advantage)**
- **May cause immune rejection & Ethical concerns (Disadvantage)**

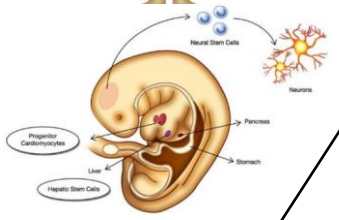




Adult Stem Cells (ASC):

- **Sources:**
  Bone Marrow, Placental Cord* & Mesenchymal Stem cells
- **Multipotent & Limited numbers and more difficult to isolate (Disadvantage)**
- **No immune rejection & No Ethical concerns (Advantage)**
- 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**

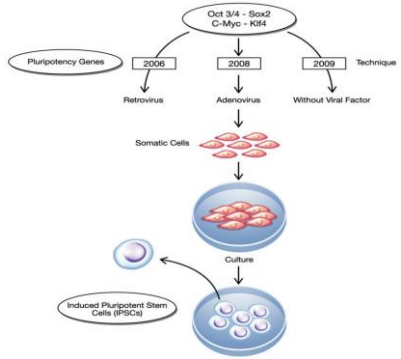




Adult or tissue specific means (multipotent, oligopotent and unipotent
ما يقصد المرحلة العمرية، يقصد الخلية نفسها كبرت

Induced pluripotent stem cells (IPSCs)

- Cells which are not stem cells but converted to stem cells
- Slides 12 & 13



Generation of embryonic stem cells (ESC)

Only on the girl's slides

Embryonic human stem cells were first isolated in 1995 by dr. James thomson.

Derived from **4-5 day old embryo** (blastocyst):

1- Trophoblast

2- Blastocoel

3- Inner Cell Mass (ICS)

Isolate and transfer of ICS into culture dish in culture media

Culture at 37c and 5% CO2

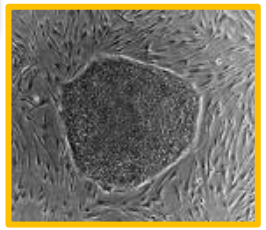
Inner surface of culture dish is coated with **inactivated MEFs** as a **feeder cells**:

- Provides sticky surface for attachment & Release nutrients

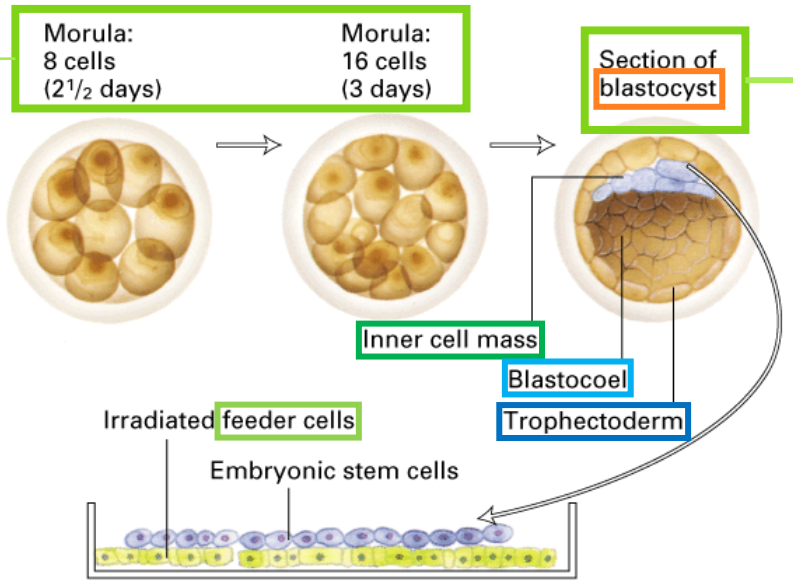
Cells divide and spread over the dish

ESCs are removed gently and plated into several different culture plates.




Totipotent



pluripotent

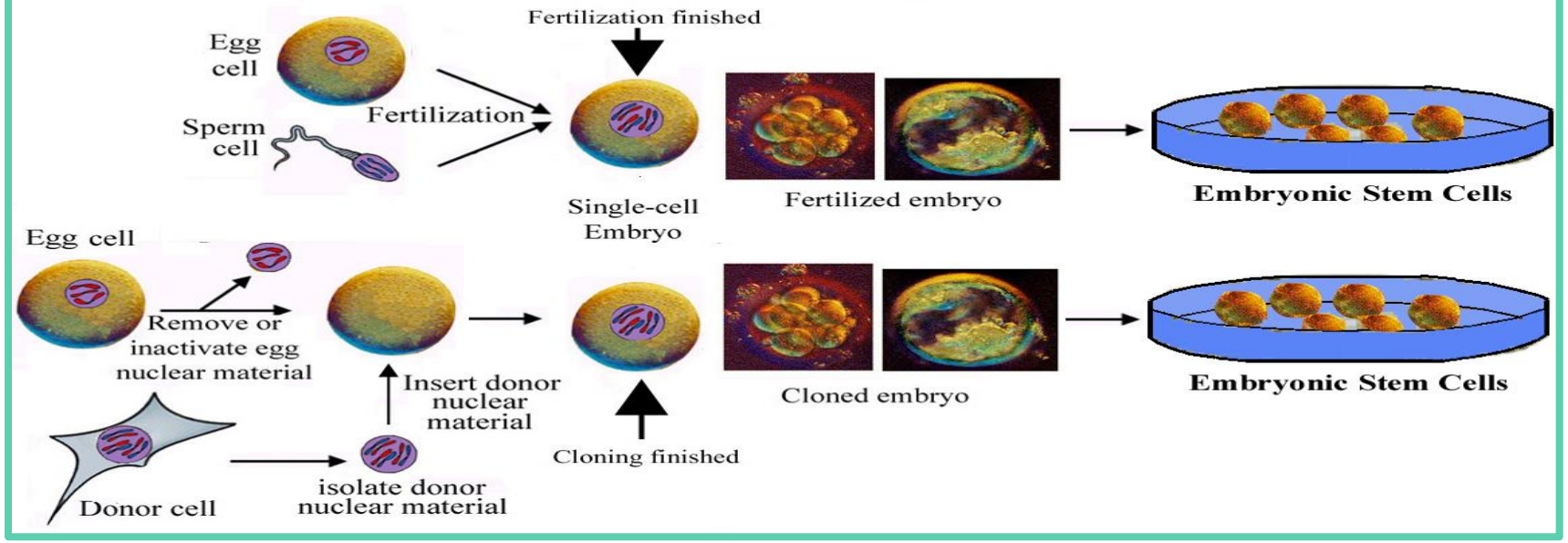
MEFs = Mouse Embryonic Fibroblasts

1- totipotent: from fertilization → implantation
2- pluripotent: it is the inner cell mass after fertilization

Only on the girl's slides

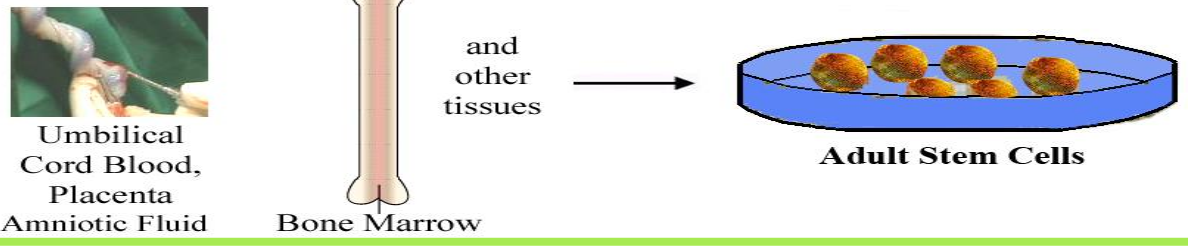
Embryonic Stem Cells

from Embryos created by Fertilization or by Cloning (Somatic Cell Nuclear Transfer)



Adult Stem Cells

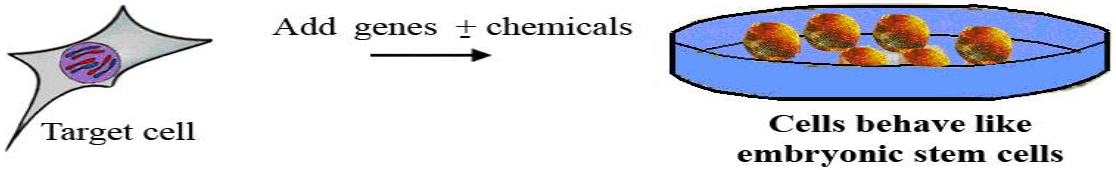
Stem Cells normally found in body tissues from birth onward, as well as umbilical cord, etc.



If there is a problem with the bone marrow, the doctors will take the mesenchymal stem cells from the umbilical cord to fix the problem.

Induced Pluripotent Stem Cells (iPS cells)

from Normal Cells that are Reprogrammed to behave like Embryonic Stem Cells



Challenges with Embryonic Stem Cells (ESC)

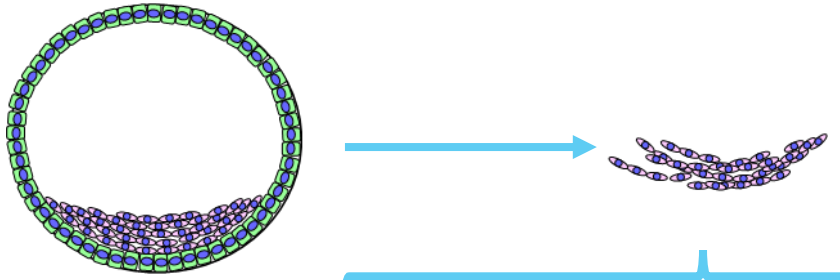
- Embryonic Stem Cells have very huge advantages: Pluripotent & unlimited ability for self-renew
- **Abnormalities** in **chromosome number** and **structure** were found in some (three) human ESC lines. (can't use them for clinical uses "treatment")
- Stem cells need to be differentiated to the appropriate cell types before they can be used clinically (if they are inserted before they are differentiated **they might multiply** and **form cancer**)
- Stem cell development or proliferation must be controlled once placed into patients (risk of teratoma formation).
- The use of mouse "feeder" cells to grow ESC could result in problems due to xenotransplantation*. *Xenotransplantation: process of transplanting tissues between organisms. (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)
- Possibility of rejection of stem cell transplants as foreign tissues is very high.

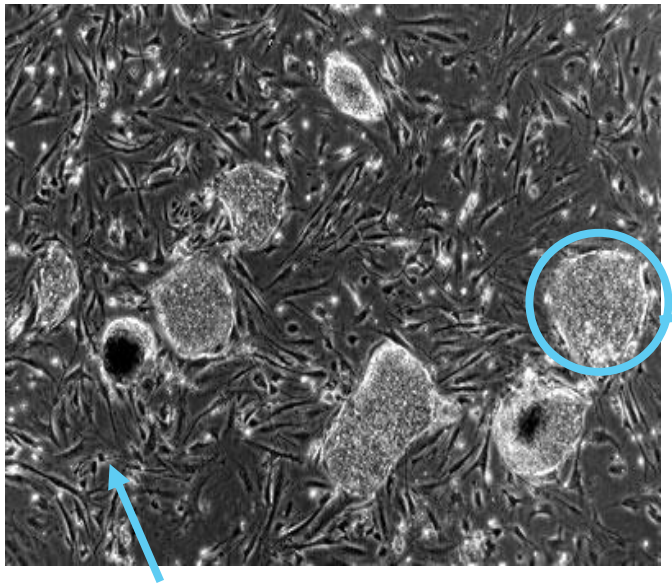
Only on the girl's slides

Human Embryonic Stem Cell (hESC) Colony

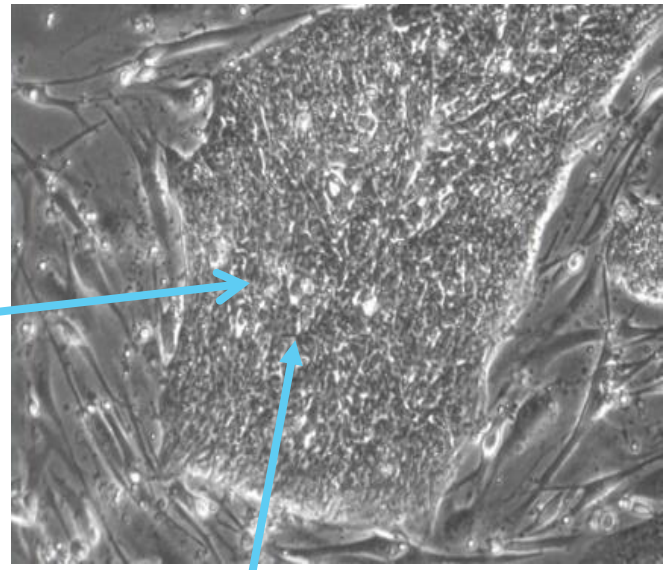
*This is a video showing cardiac cells beating.
(to view it download the ppt version)

- What do cultured ESC (Embryonic Stem Cell) look like?

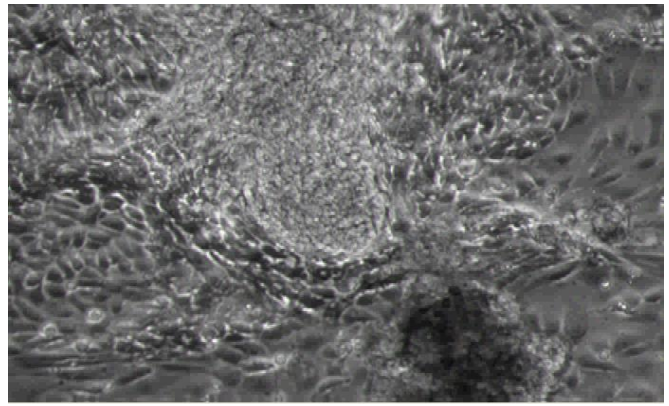




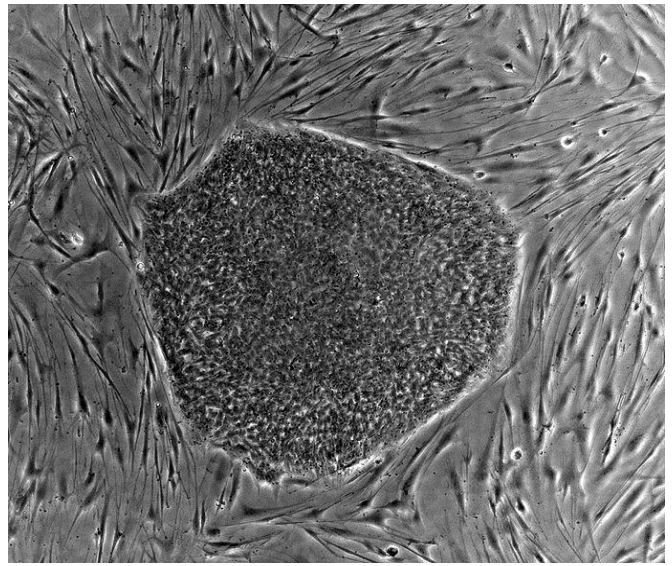
Mouse embryonic cells (feeder layer)
The lines around that surround ESC



Embryonic stem cell colony with distinct border



Beating cardiomyocytes derived from hESCs*



Embryonic stem cells in the dish

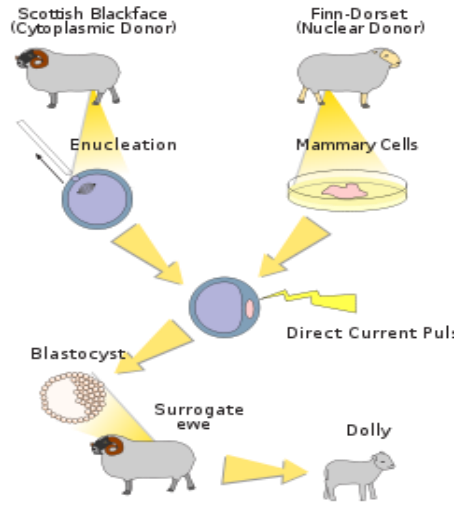
Only on the girl's slides

Somatic Cell Nuclear Transfer (SCNT) CLONING

Nuclear transfer (cloning) can be used in 2 ways: reproductive (producing identical offspring) or therapeutic (which is the main goal)

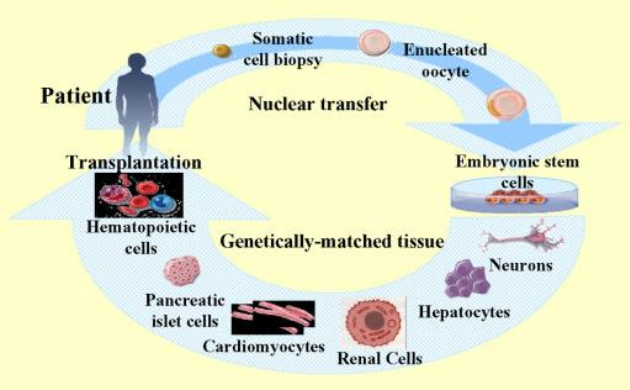
Reproductive Cloning

- **Dolly** is a sheep that was cloned from another sheep using the same method we discussed before. An oocyte was deprived of its nucleus and a different nucleus was inserted and the blastocyst was reinserted into a surrogate mother.



Therapeutic Cloning

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



The first induced pluripotent stem cells (IPSCs)

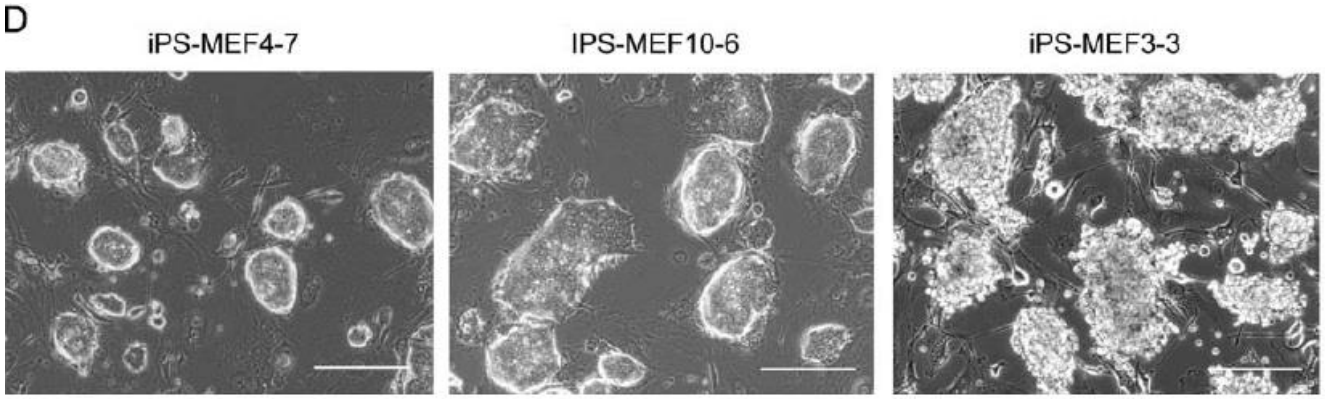
Only on the girl's slides

- 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.

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

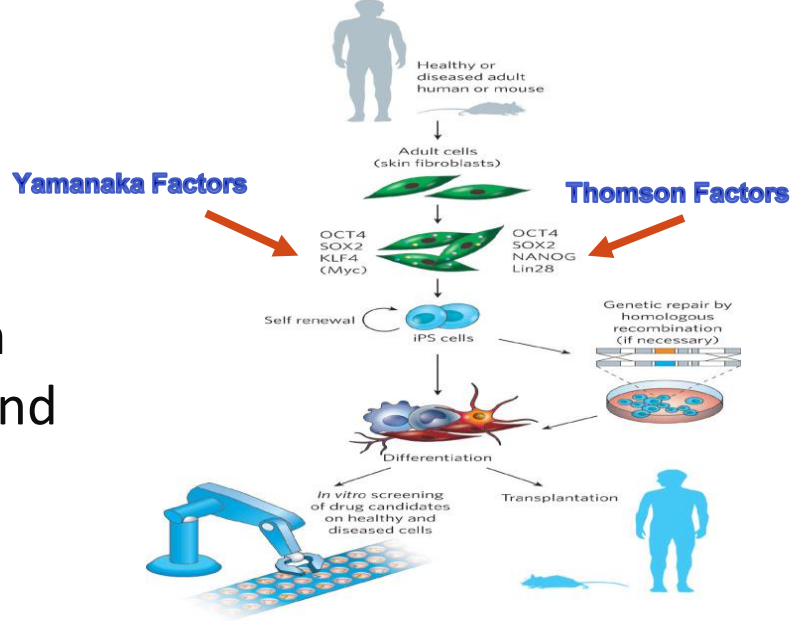
Cell

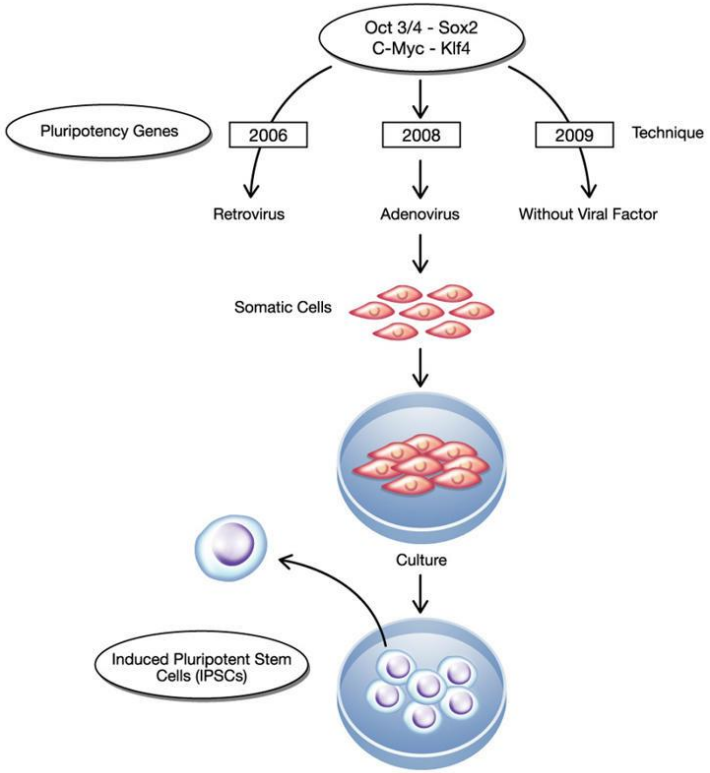
Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2]CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024



Induced pluripotent stem cells (IPSCs)

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





Induced pluripotent stem cells (IPSCs)

1st STEP

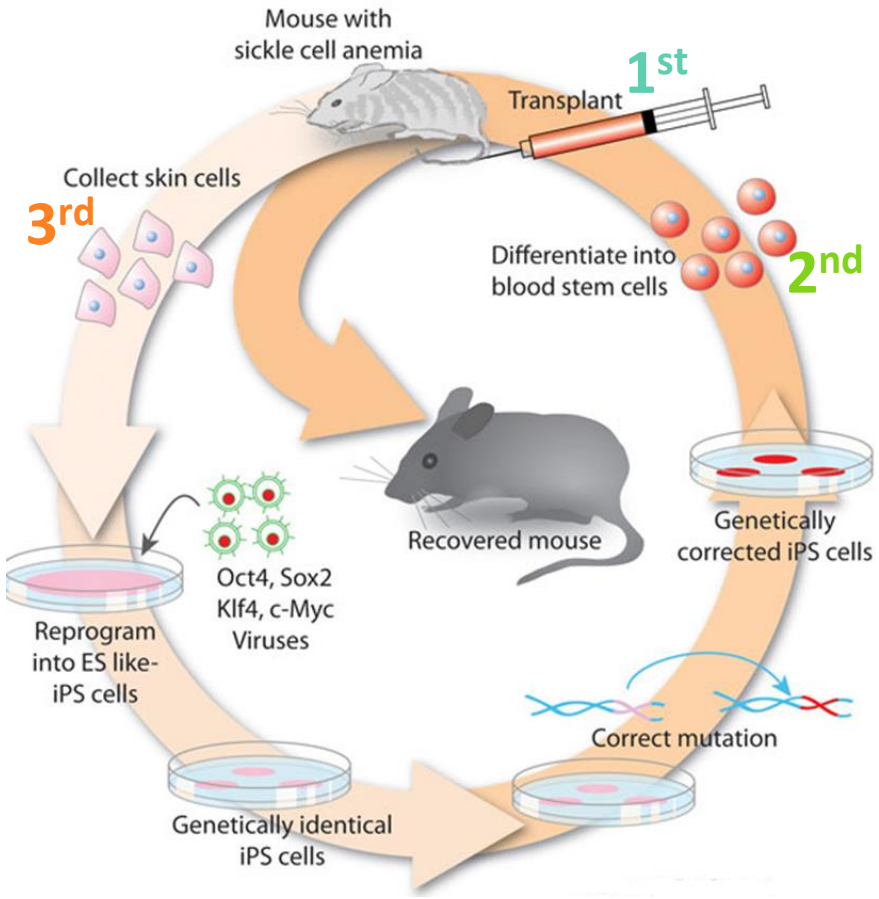
- Skin cells were taken from the tail tip of a sickle-cell model mouse

2nd STEP

- Were differentiated into hematopoietic cells.

3rd STEP

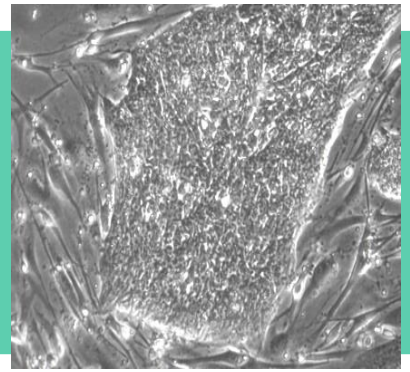
- The produced cells were transfused back into the sick mouse

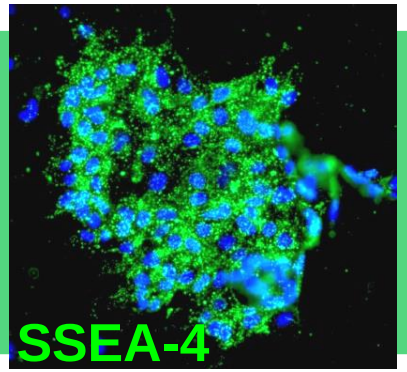


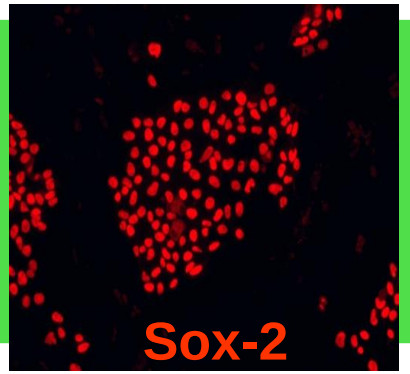
Human Pluripotent Stem cells (hPSCs) Characterization

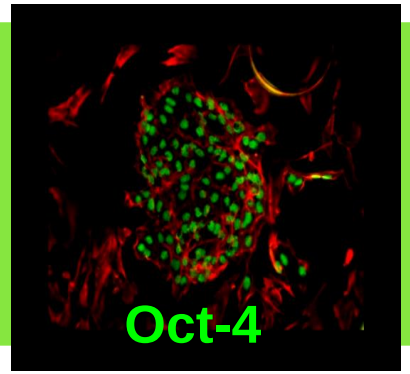
Specific markers for stem cells

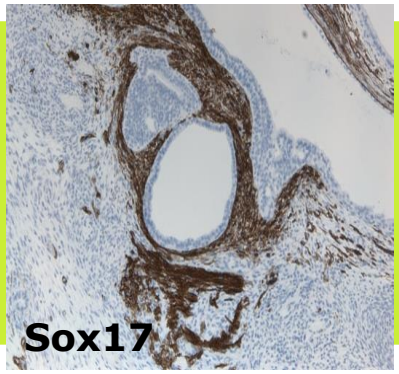
When they put these genes in a somatic cell, it becomes a stem cell.

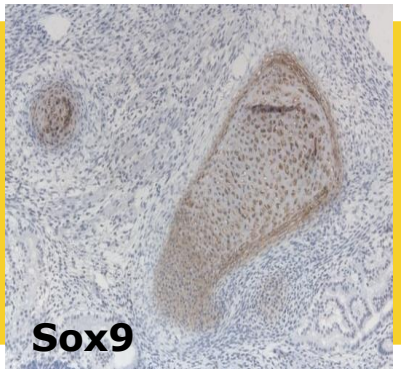




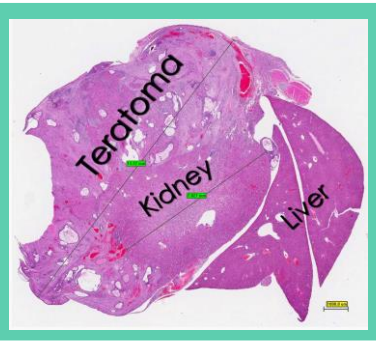




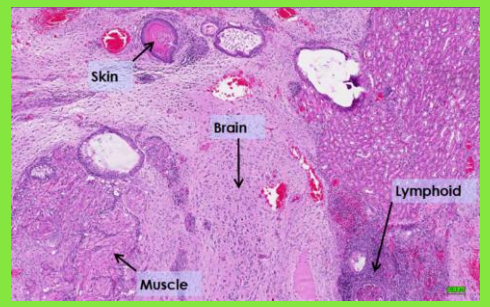




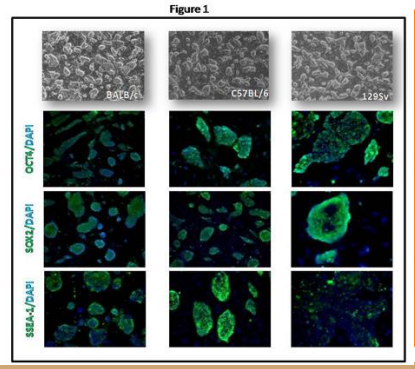
Human Pluripotent Stem cells (hPSCs) Characterization



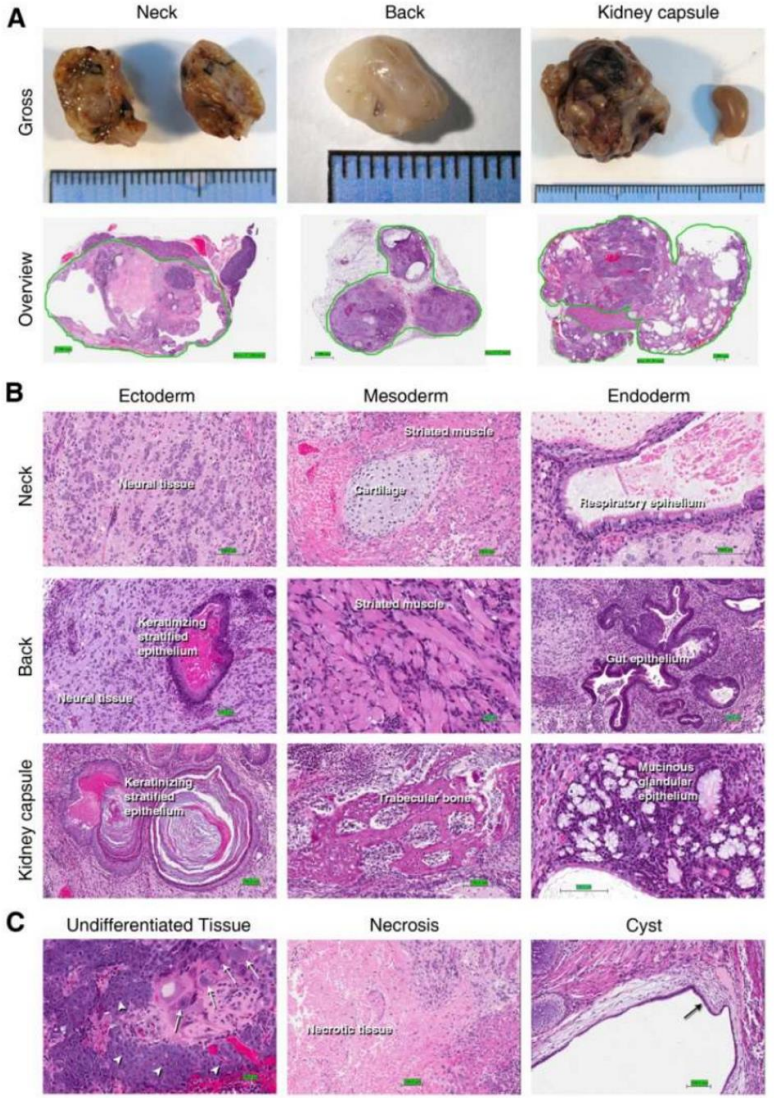
- A large tumor mass measuring twice as the kidney is compressing it.



- The teratoma was composed of mixed tissue patterns: skin with keratin, brain tissue, striated and smooth muscle, lymphoid tissue,….

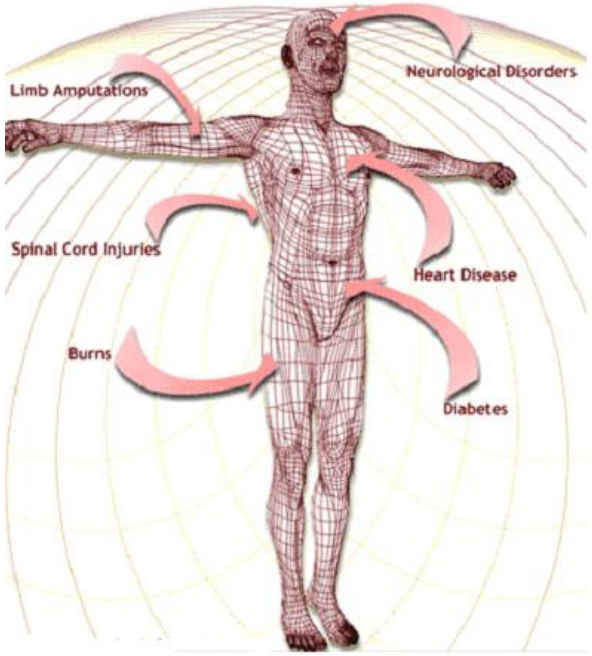


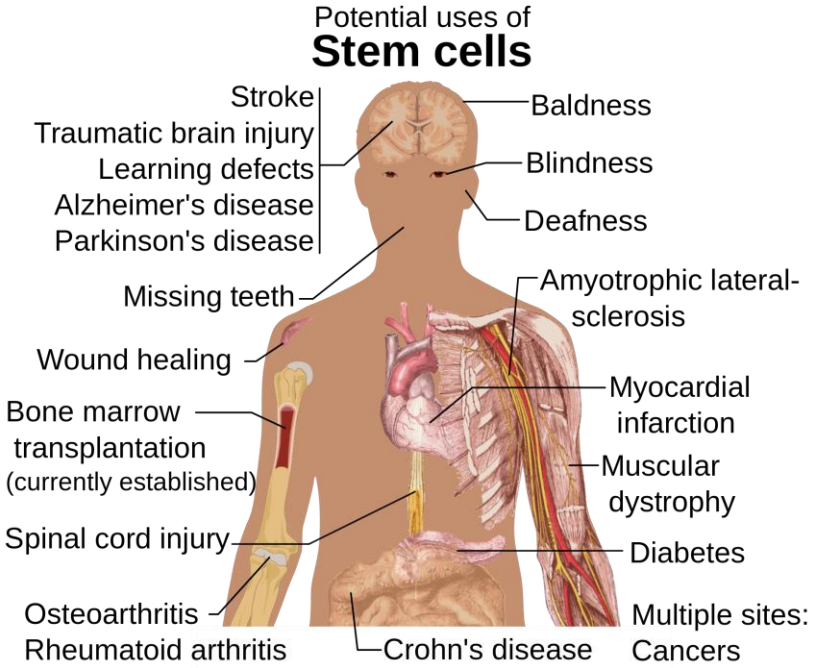
- Teratoma Formation in Immunocompetent Mice After Syngeneic and Allogeneic Implantation of Germline Capable Mouse Embryonic Stem Cells, 2013



The Promise of Stem Cell Technology

- Replacement of tissues/organs
- Repair of defective cell types
- Study cell differentiation
- Toxicity testing.
- Understanding prevention and treatment of birth defects.
- Study of development and gene control.
- Study of drugs therapeutic potential.





Only on the girl's slides

Goal of Stem Cell Therapies

- The goal of stem cell therapies is to promote cell replacement in organs that are damaged and do not have the ability for self repair (treat diseases)

Obstacles of Stem Cell Research

- How to find the right type of stem cells?
- How to completely differentiate Stem Cells to desired cell type?
- How to put the stem cells into the right place?
- Will the stem cells perform the desired function in the body?
- Differentiation protocols for many cell types have not been developed.

Summary

Stem cells	
Definition	Cells that have the ability to continuously divide and differentiate to other kinds of cells
Function	Repair and regeneration of tissues
Classification	**1.potency based** A. Totipotent : from embryonic and extra embryonic cells B. Pluripotent : form 3 germ layers C. Multipotent : form related cells D. Oligopotent : form few cells E. Unipotent : form one cell type F. Nullpotent : terminal cell **2.Sourced based** A. Embryonic (pluripotent ,may cause immune reaction) B. Adult (multipotent , no immune reaction) C. IPSCs (no immune reaction or ethical dilemma)

MCQs

(1) Which of the following is The Promise of Stem Cell Technology?

A) Toxicity testing
B) Understanding prevention and treatment of birth defects
C) Study of drugs therapeutic potential
D) All are true

(2) 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 many differentiated cell types except placenta

(3) Which of the following forms embryonic and extraembryonic cell types?

A) Totipotent B) Multipotent
C) Oligopotent D) Unipotent

(4) The Blastocyst is formed of each of the following except?

A) Trophoblast B) Morula
C) Inner Cell Mass D) Blastocoel

(5) Hematopotic stem cells gives:

A) Cells of the nervous system B) Cartilage
C) Blood cells D) Connective tissue

(6) 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

(7) Mesenchymal stem cells are example of?

A) Pluripotent stem cells B) Multipotent stem cells
C) Totipotent stem cells D) Induced pluripotent stem cells

(8) The goal of stem cell therapies is to:

A) Reduce the Possibility of immune rejection
B) Promote cell replacement in organs that are damaged and do not have Ability for self-repair
C) To make full humans
D) Non of them

(9) What are yamanaka factors:

A) OCT3/4, SOX2, KLF4, c-Myc B) Growth factors
C) Cytokines D) OCT3/4, SOX2, Nanog

(10) important limitation of using cloned ESCs (SCNT-ESCs) clinically:

A) Immune rejection
B) Produce limited number of cell types
C) Destruction of human embryos
D) Difficult to grow and culture in the laboratory

Answers

(1) D
(2) D
(3) A
(4) B
(5) C
(6) C
(7) B
(8) B
(9) A
(10) C

Good luck
Special thank for team436 ❤

Team Leaders:

Faisal Fahad Alsaif
Rawan Mohammad Alharbi

Team Members:

Abdulaziz Aldukhayel
Abdulrahman Alduhayyim
Rinad Alghoraiby
Majd Albarrak

- References:
  1.Girls' & Boys' Slides

Twitter.com/Anatomy437
Anatomyteam.437@gmail.com