CENTRAL NERVOUS SYSTEM

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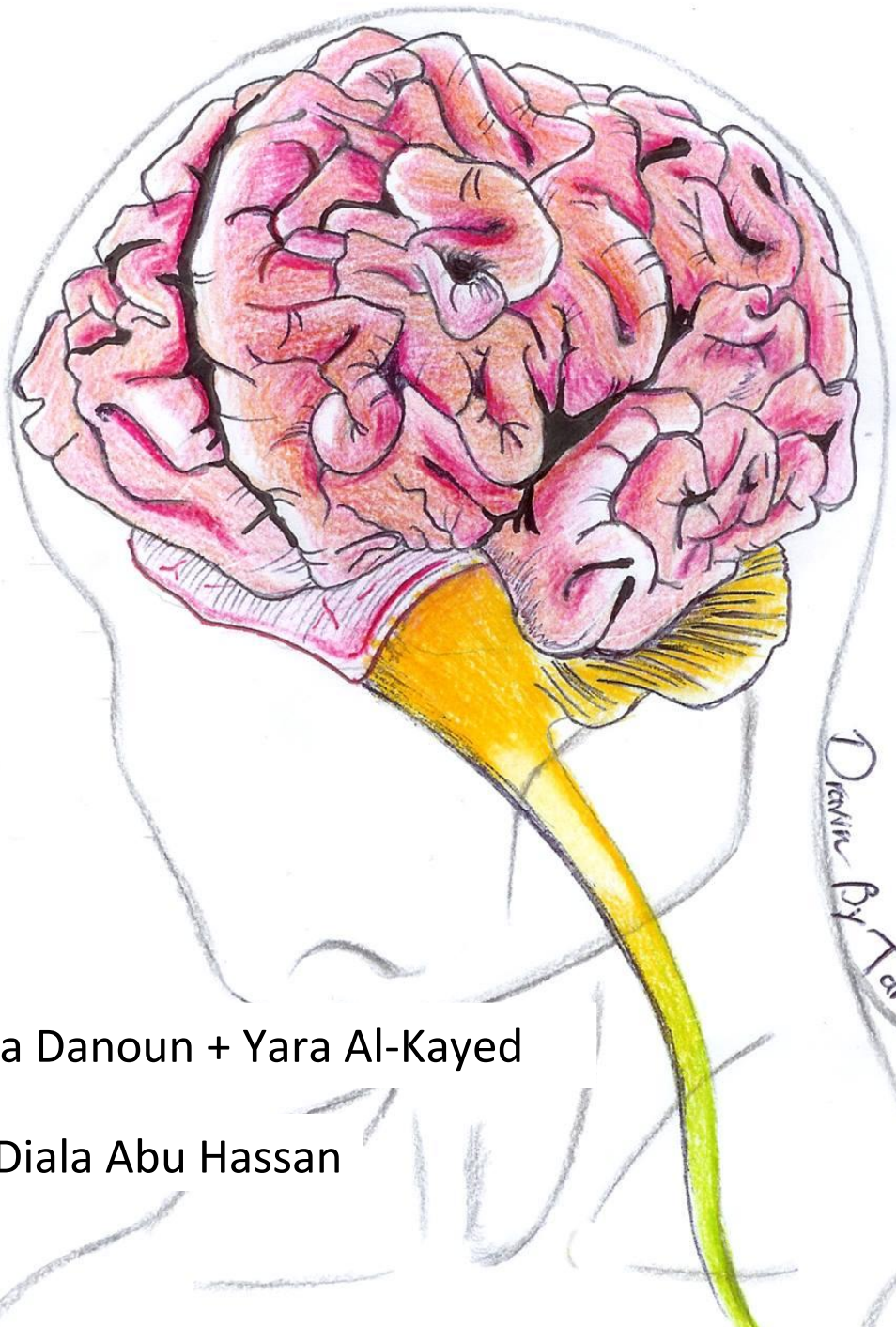

Drawn By Tariq Bushnaq...

Done By: Baraa Danoun + Yara Al-Kayed

Dr. Name: Dr.Diala Abu Hassan

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Stem Cells

What are stem cells?

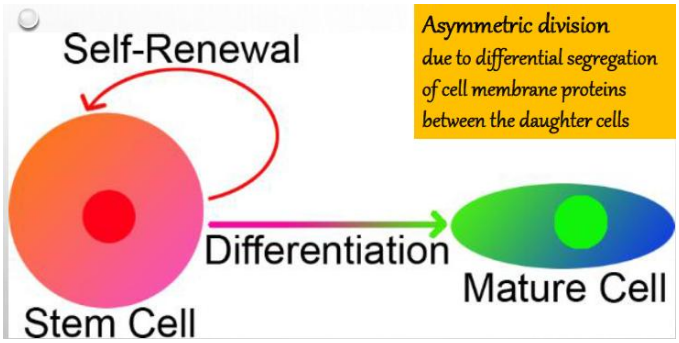
They are basically primal undifferentiated cells that have the potential to differentiate to several cell types depending on their potency; they also have the ability to self-renew. They're found in multicellular organisms (can't be seen in bacteria).

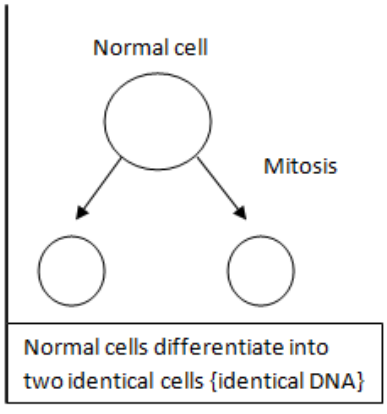
If I have a pool of stem cells they need to divide to meet 2 purposes:

1. To renew themselves in order to keep a pool of stem cells
2. To give rise to new cells types (differentiated cells)

Lineage: is a pool of stem cells that are going to differentiate to very similar cell types.

Stem cell is different from any other cells. Here mitosis is **asymmetric;** it will produce two different products one of them renews and keeps stem cells pool, while the other one is going to undergo differentiation and give rise to another cell type, that's why we called it asymmetric division.





But how can I induce **asymmetric division**?

This is done through asymmetric segregation and separation of the factors (proteins) that surround the resulting cells; certain type of factors surround the cell that's going to become a stem cell while other types of factors surround the cell that is going to become a fully differentiated cell. Segregation of these proteins makes the resulting cells different from each other.

Each of the stem cell types is present in a specific microenvironment (**stem cell niche**) to keep them in their pluripotent format, as stem cells are very sensitive and almost anything can trigger their differentiation (as simple as touching them). It's important to keep the stem-ness of stem cells so we can find them when we need them especially when we talk about adult stem cells which are used for regeneration and fixation of damages which happen continuously.

Stem cell niches differ from one cell type of cells to another; some are composed of cells only, others contain cells and ECM component; such as (fibers, collagen, elastin, proteoglycans). Others may also include secreted or cell surface factors that mediate some sort of interactions which keep stem cells in a healthy situation, examples include: Notch, Wnt, TGF-B, EGF {epidermal growth factor}, SCF {stem cell factor}.

<u>Why do we need this niche?</u>

- Nutrition for stem cells in order to keep their viability.
- Feedback control : at some time I need expansion of stem cell pool (for e.g. : for person who burn his arm) I need to regenerate stem cells or in another time I need to reduce stem cell pool, so niche also helps and provides feedback control to either expand or shrink stem cell pool .
- Coordination between different compartments among tissue because stem cells aren't isolated from the surrounding tissues. Driving

differentiation or stopping it? the cell will differentiate into several cell types which one should be on now and which one should be off? this will be coordinated in the presence of niche.

- Acting as a hub for inter-lineage coordination :

What does hub mean? نقطة موزّعة a distributor point that gives off differentiated cells upon need.

Stem cells can be divided into two general types:

1) Embryonic stem cells → For fetal development

2) adult stem cells → For Regeneration of normal tissue types (e.g. gut mucosa) and for healing of damaged tissue

The main difference between these two types is **the level of Potency**

Potency: it's the number of cell types one type of stem cell can differentiate into (differentiation potential).

According to potency stem cells divided into:

1. Totipotent:

Found at the fetal four cell stage (Morula).

Cells can differentiate into:
- All embryonic cells and tissues
- Extraembryonic structures or tissues (ex: placenta).

2. Pluripotent:

After the 4 cell stage, the fertilized ovum is going to continue its division until it reaches the next stage which is the formation of Blastocyst which is a ball-like structure, it's hollow from inside and lined from outside by thin layer of cells and there is an aggregation of cells on one pole called inner cell mass. Inner cell mass is where we'll find embryonic stem cells so it is the next level of potency.

These cells can differentiate to give any type of embryonic tissue, but NOT extra-embryonic tissues. They develop before implantation into the uterus.

3. Multipotent Stem cells (Adult stem cells):

They give rise to several cell types (related cell types) such as hematopoietic stem cells that give rise to all blood cells.

4. Unipotent stem cells:

They can differentiate to only one cell type.

What keeps the pluripotency of the embryonic stem cells? What keeps them away from differentiation?

- Niche
- Inhibitory factors of differentiation
- Pluripotency factors such as : Oct4, Nanog, Wnt- β-catenin KLF4

Mechanism of using embryonic stem cells for therapeutic purposes:

Sperm + ovum "from patient's parents"→ fertilized ovum→ several division → Blastocyst level → isolate the inner cell mass (source of embryonic stem cells) then you either differentiate them into the cells of interest and transplant them into the patient OR you can transplant cells directly (in their stem cell form) without differentiation relaying on the surrounding environment of the transplantation sight (organ's niche) to guide stem cells into differentiation as they will try to adapt to that environment.

For example: for a diabetic patient the embryonic stem cells need to be differentiated to β cells, then cells must be transplanted to the patient's pancreas. Eventually these cells will start secreting insulin.

Implanting already differentiated cells is better, because embryonic stem cells give rise to all cell types from all 3 germ layers so they can develop teratomas (teratoma is a tumor containing cells from all 3 germ layers), so instead of helping a patient in need, you put him at risk for cancer. That's why implanting fully differentiated cells is safer.

The ethical dilemma of embryonic stem cells:

It's really hard to balance two things:

1) Helping people who are suffering.
2) Respect the value of human life, if we consider the blastocyst stage of the embryo as a human being, so we'll be killing a human life.

Morals and religion are the major players in this dilemma. If we consider religion; Christianity does not accept this at all, because in Christianity once fertilization occurred it's considered a human life. On the other hand, in Islam and Judaism, soul is considered to be given to the embryo after 40 days of gestation نفخ الروح . Others may consider that before implantation in the uterus the fertilized egg is not considered as a human being, and implantation occurs at day 14, so before day 14 they allow embryonic stem cells usage for research purposes. Usually the cells are used at day 5.

The debate will never end as people have different believes, so it's like a personal issue whether to use them or not.

Induced pluripotent stem cells (iPS):

To solve the problem of embryonic stem cells and its associated ethical dilemma, a Japanese scientist Yamanaka who won a prize for his invention back in 2012 obtained pluripotent stem cells from differentiated cells.

He took a skin biopsy isolated the fibroblasts, de-differentiated them so they became pluripotent stem cells and then he could differentiate them to any cell type of all 3 germ layers. These are called induced pluripotent stem cells (iPS).

Two years after, a scientist in UK was able to make induced pluripotent stem cells from a urine sample as he considered a skin biopsy to be invasive and he wanted to find a more benign way to get these cells.

Advantages include:

- In this way, we'll avoid the ethical dilemma,
- These cells are autologous (from the patient himself) because embryonic stem cells may not match 100% with patient (siblings don't match 100%)
- patient specific therapy
- Safer as they're autologous we expect the rejection to be reduced.

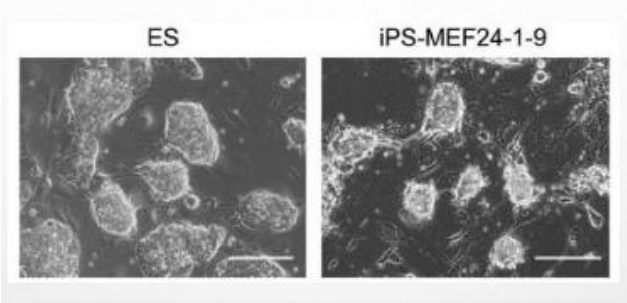
How did Yamanaka generate these iPS? How did he induce de-differentiation?

He used the pluripotency transcription factors and expressed them inside these cells, once these genes are turned on; they converted the cell into a pluripotent stem cell.

There are around 24 pluripotency transcription factors, Yamanaka at first turned all the 24 TFs on, and then he tried to turn-off one TF at a time in order to be able to get iPS with the least number of turned on genes.

He could at the end get iPS with only 4 pluripotency TFs which are: **OCT3/4, SOX2, c-Myc, KLF4**. If you look carefully at this set, especially the Myc which is an oncogene if it's highly expressed it may cause cancer that's why several trials followed trying to change some of these TFs to safer ones and avoid expressing oncogenes.

Yamanaka's comparison between iPS and ES:



Yamanaka has found that iPS and embryonic stem cells are very similar in many aspects, such as:

- Morphology: both grow in colonies close to each other (you don't find single cells), but morphological similarity alone isn't enough.
- Surface antigens: membrane proteins were very similar.
- Gene expression profiles (which genes are highly expressed) were also similar.
- Telomerase activity: iPS are acquired from adult cells which are old so we'd expect the telomeres to be short, however, telomerase is highly active in iPS as it is in ES to lengthen the telomeres. It's better to use adult cells that are not very old to have a better treatment potential.

- Epigenetic status: factors that either inhibit suicidal or activate cell genes were very similar.
- Promoter activity: "Promoters are located near the transcription start sites of genes" -wikipedia.
- Proliferation potential: frequency and amount were similar.
- In vitro differentiation: the ability to differentiate to cells of three different germ layers.
- Teratoma formation: as iPS are able to differentiate to all cell types of all 3 germ layers, they are able to form teratomas (tumors containing cells from all 3 germ layers).

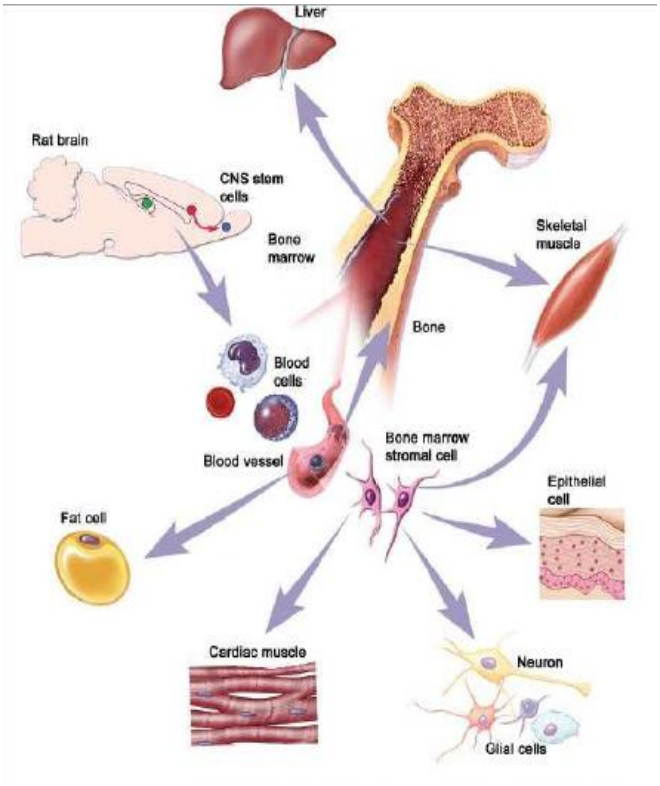
Adult stem cells:

They are mostly concerned with regeneration and healing of body tissues.

Types:

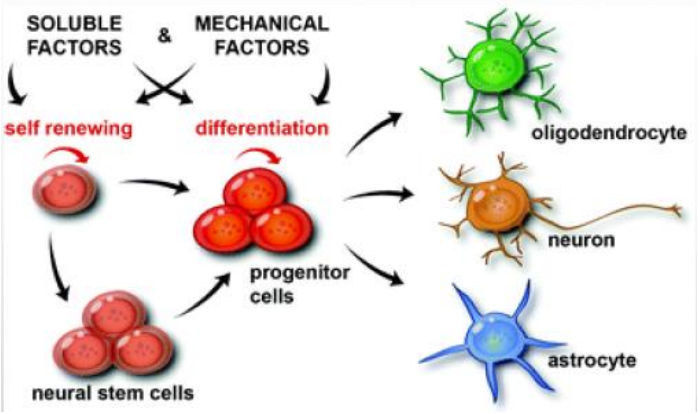
1) Bone marrow stem cells:

A. Hematopoietic stem cells: for formation of different types of blood cells (WBCs, RBCs, Megakaryocytes → platelets).
B. Somatic stem cells, such as:
    - Mammary stem cells that are activated at puberty for the formation of all components of mammary glands.
    - Mesenchymal stem cells (osteoblasts, chondrocytes, myocytes, adipocytes, neuronal cells) that give rise to bones, cartilage, muscles, adipose and neuronal tissues respectively.



2) Neural stem cells:

They're found in different regions specifically in the hippocampus, they regenerate neural cells as well as oligodendrocytes and astrocytes.

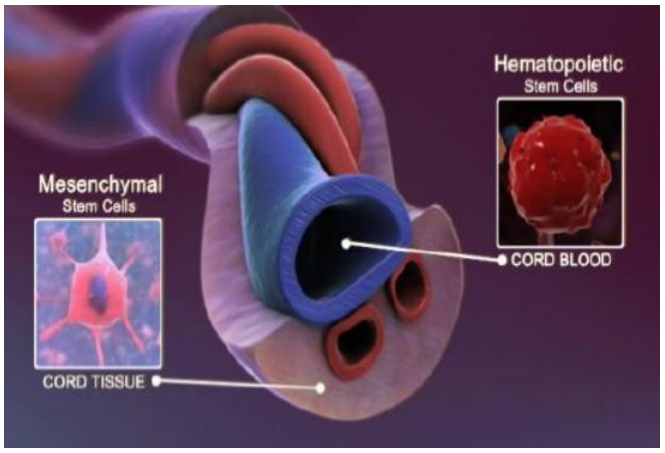


3) Adipose stem cells (ASCs):

They can be isolated from adipose tissue after liposuction, cultured in dishes and differentiated to different cell types. These cells are very similar to mesenchymal stem cells in terms of origin, so they can give rise to fat cells as well as mesenchymal stem cell derivatives.

4) Umbilical cord stem cells:

A. Hematopoietic: inside blood vessels of umbilical cord.
B. Mesenchymal: part of the cord tissue itself.



In Jordan we have a center called "Baby Cord" where newborns' umbilical cords can be stored in liquid nitrogen as a future reserve.

These cells are considered as multipotent stem cells and not pluripotent, only embryonic stem cells or iPS are pluripotent.

5) Olfactory adult stem cells:

These cells are found within the olfactory epithelium and olfactory nerve and can regenerate both olfactory nerve cells and olfactory epithelium.

6) Tissue stem cells:

They're found in many sites throughout the body (in localized tissue), examples:

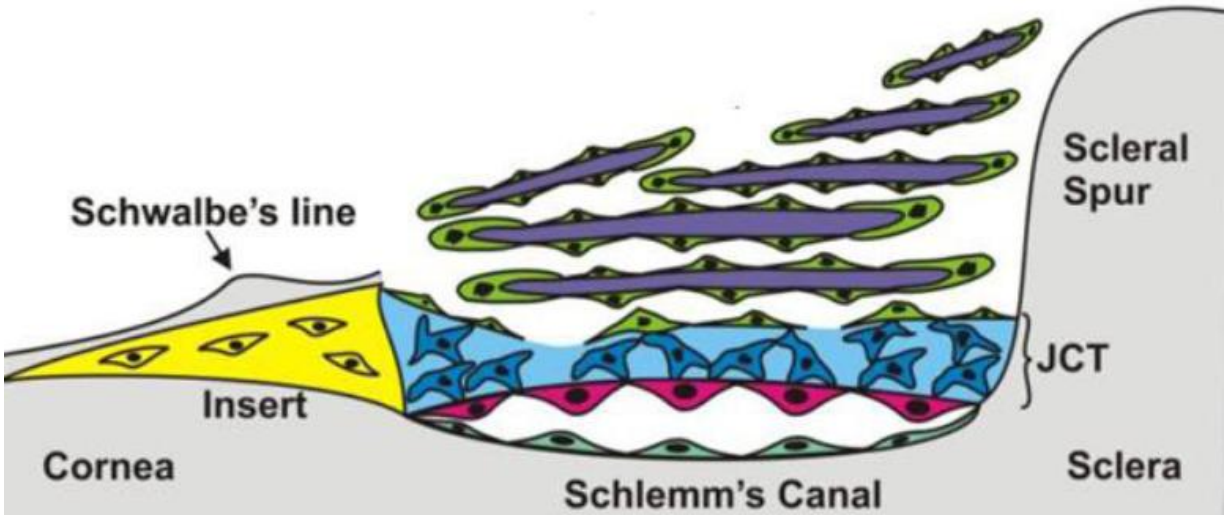
- Cornea.
- Intestinal cells: intestinal stem cells are needed due to the high frequency of intestinal cell regeneration "every 3 days".

- Trabecular meshwork cells in the cornea (insert cells):

  If we take a side view of the eye we'll find cornea in the front followed by the iris, these two structures meet at an angle called Iridocorneal angle, here we find the trabecular meshwork which is the tissue responsible for intraocular pressure regulation by filtration of aqueous humor and allowing its exit into the venous circulation

  Insert cells are the stem cells that regenerate cells of the trabecular meshwork. These are found at the Iridocorneal angle under the Schwalbe's line where we find Insert cells inserted into the cornea.

  To treat a patient with glaucoma where intraocular pressure is raised, we can induce damage (burns) to the trabecular meshwork cells by laser, thus activating insert cells to heal damaged tissues and produce new normally-acting trabecular meshwork cells.



Trans-differentiation vs developmental plasticity:

- Trans-differentiation: A change in stem cell differentiation from one cell type to another → NOT a feature of stem cells.

- Developmental plasticity: the ability to differentiate into different cell types (multiple differentiation options) → a feature of stem cells.

Why stem cell research is important?

- Cell-based therapy and regenerative medicine, which is a better alternative for using drugs.

- Drug testing: as people respond differently to same drugs -because of genetic variations- we can use stem cells to test the patient's response to the drug before administrating it especially in cases of cancer chemotherapy. For example, to treat a leukemic patient, we can take a fibroblast from him → de-differentiate it into a pluripotent stem cell → induce its differentiation into a WBC → administer the drug → check for response → if response is proper → administer the drug.

- Creating human disease models (modelling diseases): same disease can have different behavior in different people-because of genetic variations-. So we can –for example- take any cell from a diabetic patient → de-differentiate it into iPS cells→ induce differentiation into pancreatic beta cells, then we can test the patient's specific response to any intervention (adding a certain molecule, activating a certain pathway) so we can understand both the pathogenesis and progression of the disease.

- Functional genomic studies: epigenetic modifications differ from one person to another, even identical twins may have different gene expression, and all of this can be tested using stem cells.

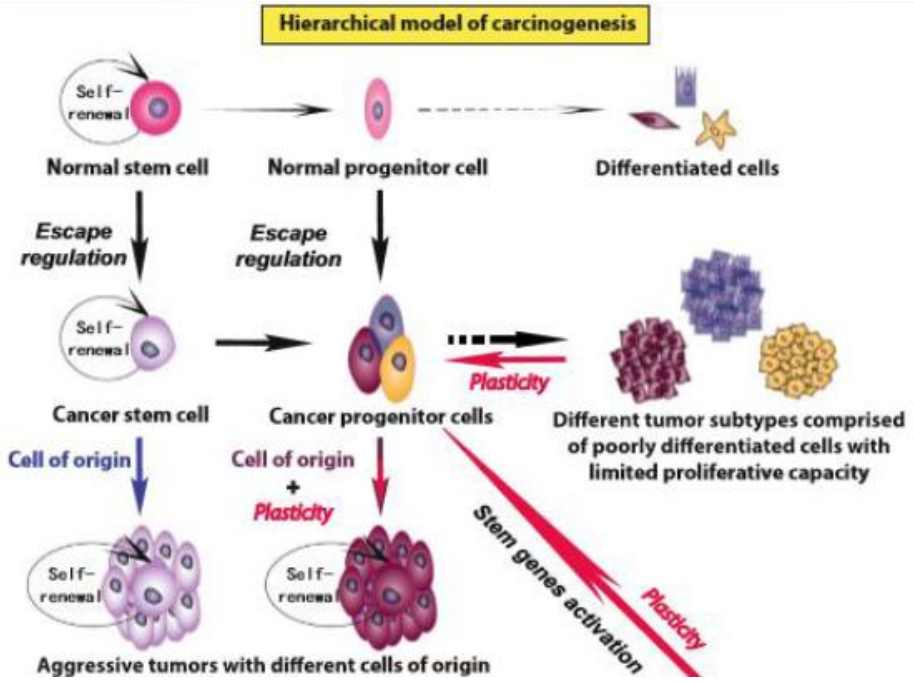
Cancer stem cells:

One of the theories studying cancer is the Hierarchical model of carcinogenesis; it suggests the presence of cancer stem cells within a tumor. Within a tumor these cells are able to self-renew and give rise to new tumor cells, which is basically the behavior of regular stem cells.

Their presence makes the tumor much more aggressive in comparison with tumors that contain only fully differentiated cells. However, in tumors containing fully differentiated cells, some of the cells can de-differentiate back into cancer stem cells.

These cells originate from:

- Early stage: Normal stem cells that get modified by a stimulus (activation of a certain oncogene, exposure to certain carcinogens "radiation, pollution") resulting in cancer stem cell formation that can progress into a tumor.
- Later stage: Normal stem cell that gets differentiated into a precursor multipotent cell, and again a stimulus can convert these precursor cells into cancer stem cells.



The End!