CANCER STEM CELL BIOLOGY and CANCER VACCINES (2)

Assoc. Prof. Pınar BAYDIN

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I. Stem cell definition

II. Cancer stem cell theories, Cancer stem cell definition

III. Stem/Cancer stem cell surface markers and isolation

I. Stem Cell Definition

Fundamental Stem Cell Properties

- "**Self-renew**": divide to produce more stem cells

- Self-renewal is defined as a special cell division that enables stem cells to produce another stem cell with the same replication potential. Self-renewal occurs as a response to systemic or local signals that induce cell proliferation while maintaining tissue-specific properties.

- Self-renewal is a cell division in which one or both of the resulting daughter cells remain undifferentiated and retain the ability to give rise to another stem cell with the same capacity to proliferate as the parental cell. Proliferation, unlike self-renewal, does not require either daughter cell to be a stem cell nor to retain the ability to give rise to a differentiated progeny.

I. Stem Cell Definition

Fundamental Stem Cell Properties

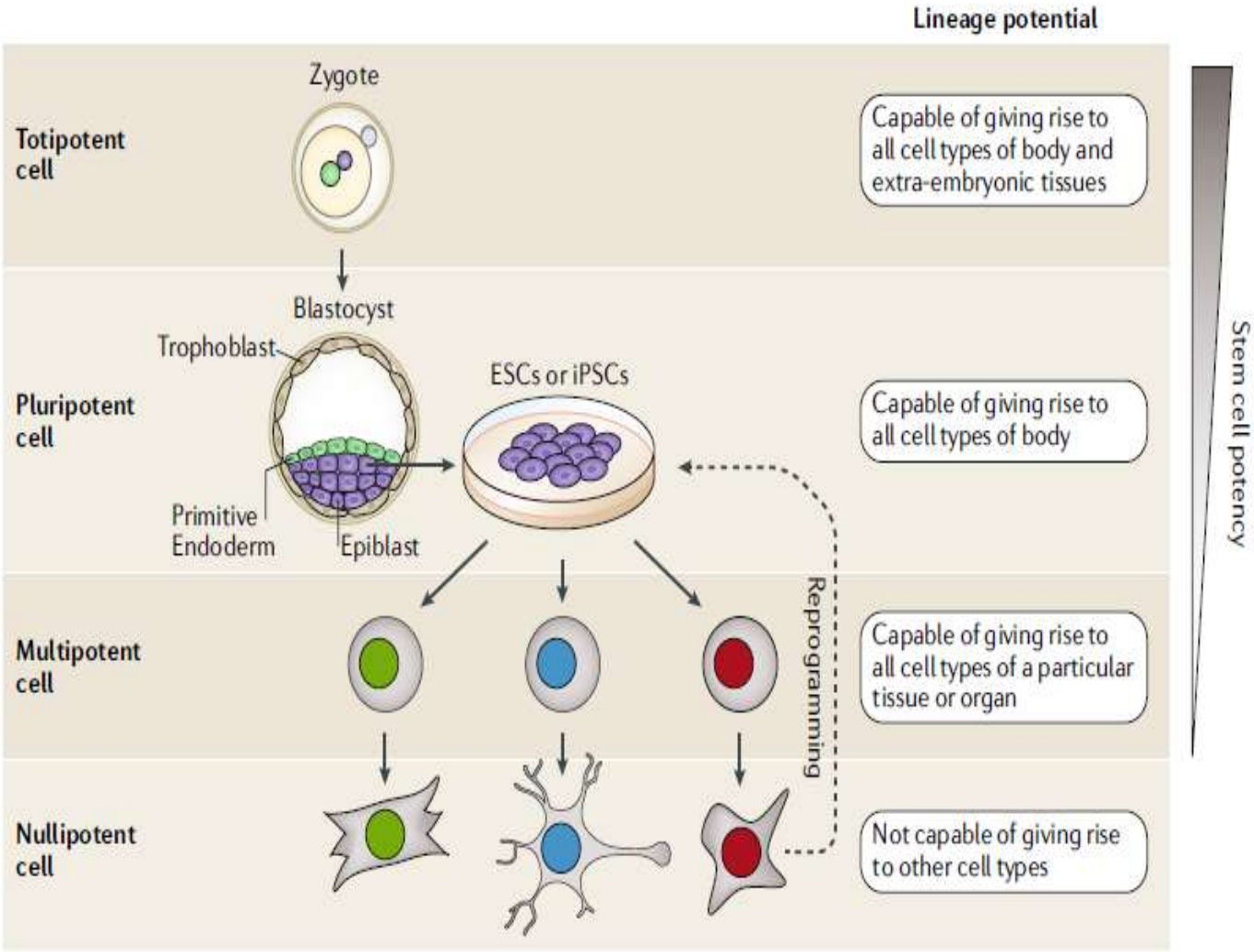
- "**Differentiation**": the ability to differentiate to give rise to specialized cells

- SSCs involves cell differentiation of daughter cells into tissue-specific specialized cells, i.e., mature cells of a specific tissue. SSCs repair damaged tissues and maintain normal tissue homeostasis by replenishing many cells throughout an organism's life.

Stem cells can be classified according to their differentiation potential:

- **totipotent** (they can generate all cell types in the body including extra-embryonic tissue or placenta),
- **pluripotent** (they can generate all body cells including germ cells),
- **multipotent** (they can be further specialized in the tissue), and
- **unipotent** (they only generate a single cell type) .

Depending on the stage of embryonic development that the embryonic stem cells (ESCs) are derived, these can be totipotent or pluripotent , while adult or SSCs are shown to be multipotent.



Tewary et al., 2018

Roles of stem cells in a biological system

- The basic biological significance of adult stem cells is to act as a source of progenitor cells which can in turn act as a repair system, primarily for that particular tissue, or other tissues of that particular germline.
- The stem cell is an essential component of a developmental phenomenon-one of the key components of a program fundamental to organogenesis and maintenance of homeostasis throughout life.
- ES cells not only can differentiate into all cell types of the body, but also contribute to maintain the normal turnover of regenerative organs such as blood, skin or intestinal tissues.
- In post natal period of mammalian development, a very small portion of pluripotent stem cells reside in all the tissues, in the form of *Very Small Embryonic/ Epiblast like Stem Cells (VSEL)*, in addition to, the uni/multipotent adult or somatic stem cells. VSEL are progenitors of most of the adult stem cells in the mammalian system.

Roles of stem cells in a biological system

- The stem cells, thus, confer plasticity to the process of growth, development and maintenance.

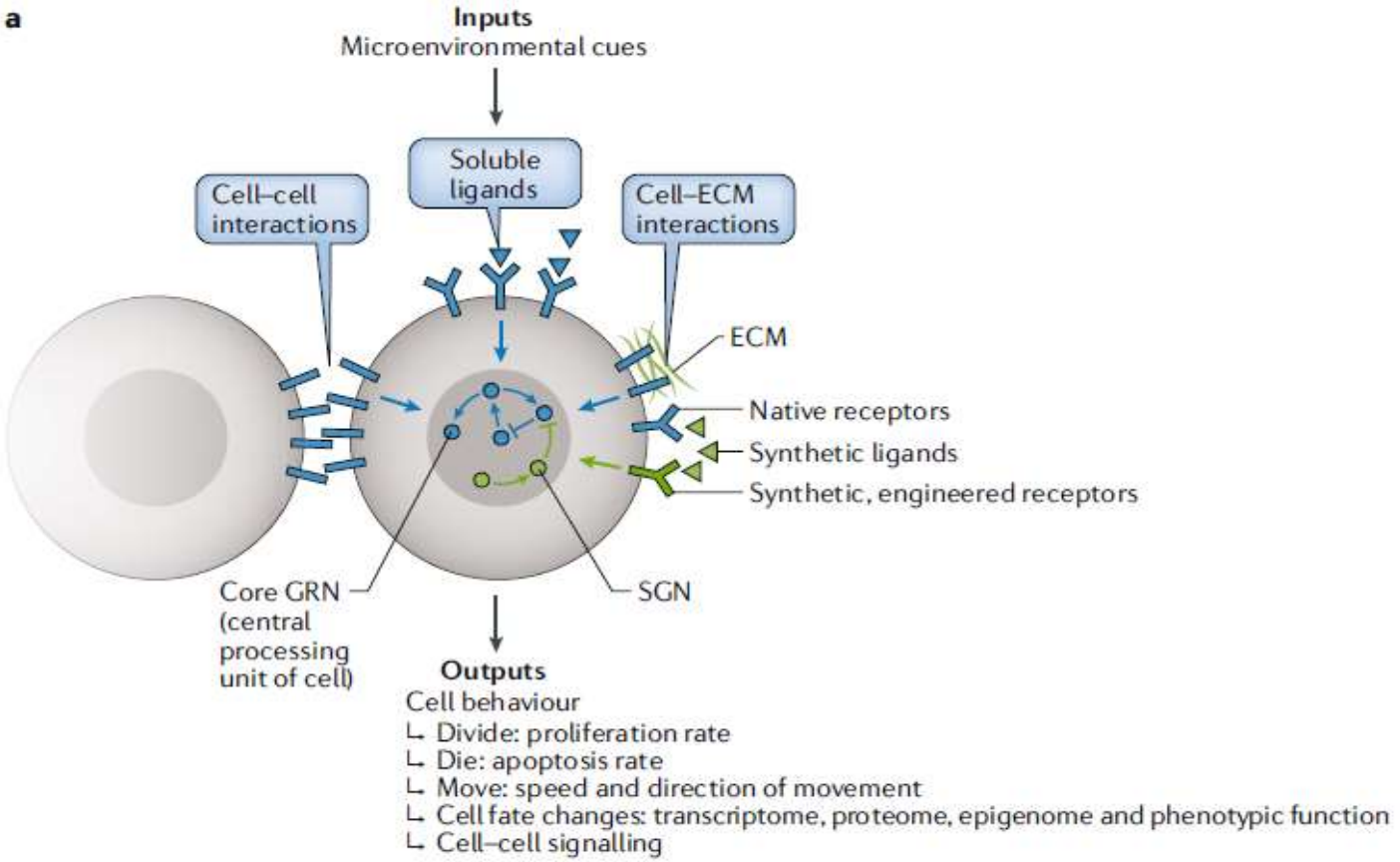
- For the reason that stem cells are a main player in the maintenance of a living organism, an understanding of the stem cell is essential for disease, aging and some of the degenerative conditions.

Information Flow in Stem Cell Systems

- Stem cells receive cues from the microenvironment in the form of both *biochemical* and *biophysical* signals.

- Biochemical signals include *autocrine* signals that are secreted and subsequently received by the same cell as well as juxtacrine and paracrine signals that are received from adjacent or neighbouring cells.

- Biophysical signals are mediated by cell–cell contact and by interactions with the shape, topology, compliance and composition of *extracellular matrix* (ECM) proteins.



Tewary et al., 2018

Stem Cell Biology Layers; *Complexity!*

- Stem cell **gene regulatory networks** (GRNs):

  To understand the stem cell behaviour, it is necessary to first uncover the key genes involved in these decision-making parts, how these regulatory players interact with one another and how they are connected to signals in their surroundings.

GRN: A set of genes and their direct and indirect regulatory interactions with one another. GRNs are like a server that process input signals and generate outputs in cell behaviour.

Stem Cell Biology Layers; *Complexity!*

- Stem cell **niche** stem cells in vivo are 'housed' in microenvironments called niches.

These niches are composed of a complex combination of factors that include supportive cells that provide appropriate signals to stem cells via cell–cell communication; the surrounding ECM, which can vary widely in terms of composition, geometry and compliance; other sources of mechanical stimuli; and physiological factors such as oxygen and pH.

Niches: The in vivo microenvironments in which stem cells reside that regulate their homeostasis and fate choices.

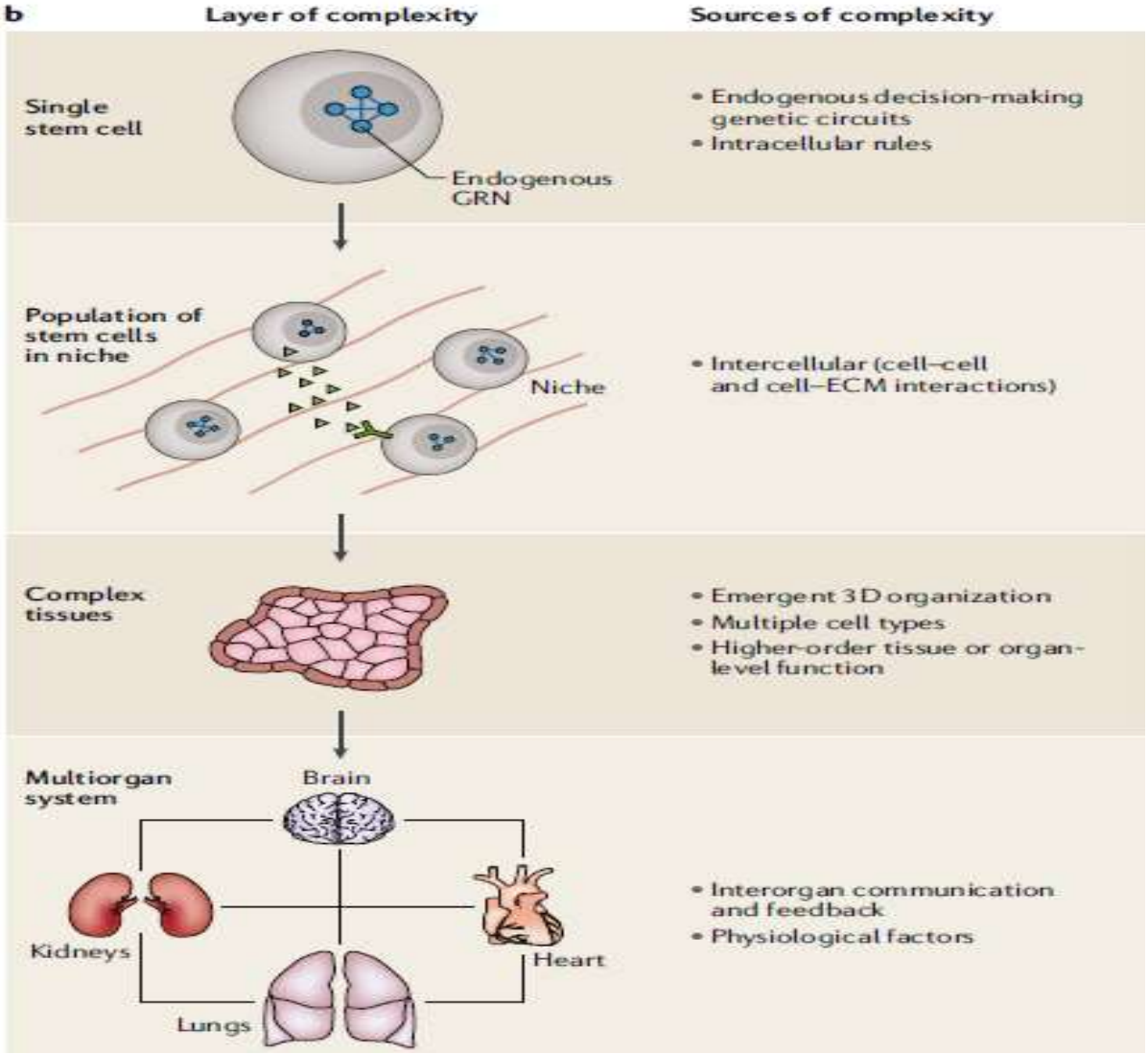
Stem Cell Biology Layers; *Complexity!*

- **Stem-cell-derived tissues and organs:** *Higher-order* stem-cell derived cell populations such as organs and tissues are highly complex and consist of multiple different cell types ordered in a spatially defined manner. These cell populations emerge from and are maintained by intercellular communication between the cells that make up the multicellular system.

Morphogenesis: The process by which developing organisms acquire their structure and shape.

Stem Cell Biology Layers; *Complexity!*

- **Interactions between stem-cell-derived tissues and organs***:* *In vivo, stem-cell-derived tissues and organs* interface with a complex environment where communication between distal organs via cell-secreted factors (for example, proteins or hormones) has a crucial role in maintaining homeostasis.



Tewary et al., 2018

The Origin of Cancer Cells

- Cancer is described as a proliferative, invasive, and metastatic disease that is caused by an accumulation of genetic abnormalities that randomly produce a malignant cell.

- Cancer originates when normal cells accumulate DNA mutations over time and lose the ability to grow and proliferate in a regulated manner, leading to abnormal cell proliferation.

- Cancer develops by the accumulation of mutations in genes leading to the deregulation of signaling pathways that initiate the gain of self-sufficient growth signals leading to insensitivity to anti-growth signals, evasion of apoptosis, unlimited replicative potential, sustained angiogenesis, and capacity to invade surrounding tissues.

Specific examples to the origin of cancer, using specific cancer types are:

i. Field theory: Teratocarcinomas arise from normal germinal cells when these cells are placed in a tissue niche that does not enforce normal differentiation.

ii. Chemical carcinogenesis: Chemicals may cause cancer. Exposure of the skin to chemical carcinogens causes mutations.

iii. Virus infections: HPV virus infects basal stem cells of the cervix and redirects the cells from differentiation to proliferation. Hepatitis virus, which infects mature liver cells, stimulates proliferation and causes maturation arrest at a late stage in the liver cell lineage.

Specific examples to the origin of cancer, using specific cancer types are:

iv. Mutations: Translocations in myeloid leukemia produce fusion proteins that are activated at various stages of myeloid hematopoiesis, leading to accumulation of cells at a specific stage of differentiation. Another example related to mutation of a specific gene Adenomatous polyposis coli (APC) is colon cancer. The sequence of events in colon carcinogenesis begins with a mutation in *APC* gene that result in a block in differentiation and in continued proliferation of colonic stem cell progeny.

v. Epigenetic changes: Sun damage predisposes skin to the development of cancer, because of a loss of expression of *p53,* via hypermethylation. Another example of epigenetic change is gastric cancer in which *H. pylori* infection of the stomach causes hypermethylation of the DNA of gastric mucosal stem and progenitor cells, loss of tumor suppressor gene function, and development of gastric cancer.

- Such features, which make a cell cancerous, are:

✓ unlimited proliferation, even in the absence of extracellular signals,
✓ resistance to apoptosis,
✓ evasion of anti-growth signals and immune destruction, and
✓ increased cellular motility, which give the added advantages to the cancer cells to metastasize.

On the other hand,

- stem cells are also resistant to apoptosis.
- stem cells are not involved in biologically destructive phenomenon like immune destruction.
- Some of the stem cells like mesenchymal stem cells have increased cellular motility, and they can also assist other cell types to have an enhanced cellular motility.
- Unlike the cancer cell motility behavior, the natural purpose of stem cell motility is secondary homing to contribute to cell and tissue regeneration.

So, stem cells and cancer cells have many of the features in common, but the purpose of such features is constructive or regenerative, in case of, stem cells while the purpose of the same features is destructive, in case of, cancer cells!!

Cancer Stem Cell Theories

- The strongest evidence for the CSC theory comes from studies in acute myelogenous leukemia (AML), when *Bonnet and Dick* performed serial transplantation experiments to show that only rare cells with high self-renewal capacity isolated from AML patients could initiate leukemia in murine models.

- Later the cell responsible for tumor initiation was identified by its phenotype as CD34+CD38−, a remarkably common phenotype of a normal hematopoietic stem cell (HSC).

- CSCs might represent a small fraction of the cells in the heterogeneous tumors, they likely play a fundamental role in cancer initiation, progression, and metastasis as well as in therapy failure.

CSCs are also called «tumor-initiating cells» have several important properties of SSCs:

- self-renewal,
- unlimited proliferation potential,
- slow replication,
- resistance to drugs, and
- the capacity to differentiate, giving rise to daughter cells, which make up the bulk of the tumor

Table 1. (A) Common characteristics and (B) distinguish between cancer stem cells and stem cells.

A.Common features found in normal and cancer stem cells (Bapat et al., 2010)	
01	Capacity for asymmetric divisions (self-renewal) thatproduces a quiescent stem cell and a dedicated progenitor and plays important role toward emerging a dangerous cell mass.
02	Regulation of self-renewability by similar signaling pathways (Wnt, Sonic Hedgehog, MAPK and Notch) and at the epigenetic level by BMI-1.
03	Capacity to arrange a hierarchy of cellular derivatives that includes progenitors and differentiating cells.
04	Extended telomeres and telomerase activity that increases the cellular life span.
05	Predilection for growth factor independence through secretion of growth factors and cytokines.
06	Stimulation of angiogenesis through secretion of angiopoietic factors.
07	Expression of similar surface receptors (e.g., CXCR4, CD133, a6 integrin, c-kit, c-met, LIF-R) those are either identified as stem cell markers or are associated with homing and metastases.

B. Normal stem cell properties versus CSC properties (Topcul et al., 2013)	
Normal stem cell	Cancer Stem Cell
Extensive but limited self-renewal capacity	Extensive and indefinite self-renewal capacity
Organogenic capacity	Tumorigenic capacity
Highly regulated self-renewal and differentiation	Highly dysregulated self-renewal and differentiation
Rare in normal adult tissues	Infrequent or rare within tumors
Can be identified based on surface markers	Similar types of surface markers as ordinary stem cells in the same tissue
Normal karyotype	Abnormal karyotype
Quiescent most of the time	Less mitotically active than other cancer cells
Capacity to generate normal progeny with limited proliferative potential	Phenotypically diverse progeny

Rahman et al., 2016

The CSC hypothesis has two separate but related components:

1. The first component concerns the cellular origin of tumors.

2. A second related component of this hypothesis is that tumors are driven by cellular components that display “stem cell properties.”

There is some controversy regarding the origin of CSCs:

- they may originate from normal stem cells,
- there are some CSCs that can arise from somatic cells.

- Since their origin is not clearly understood, the term **tumor-initiating cells** have been used to define these cells. In general, CSCs are considered the seed of the tumor mass which also promotes its growth

- CSCs share the same cellular and molecular mechanisms that regulate SSCs; however, CSCs lack the necessary control system to prevent uncontrolled proliferation.

- While the specific origin of CSCs is still under debate, evidence suggests that **they originate from stem cells that failed to control proliferation under abnormal circumstances**.

- Other proposed origins suggest that **CSCs could arise from cell-cell fusion between cancer cells and adult stem cells, gene transfer between somatic and cancer cells, or mutations in stem cells**.

- In addition, **transformation could occur during the process of tissue regeneration in response to inflammation, infection, toxin exposure, and/or metabolic processes which could cause mutations**.

- Consensus has not yet been reached on the criteria for classifying CSCs and therefore it has not been possible to definitively define the proportion of CSCs subpopulation in a given tumor, the relevance of CSCs to clinical outcome, and the origin of CSCs.

- Initially, CSCs were believed to represent a small fraction of the total cell population in a solid tumor, however, it has been claimed that **as many as 25% of cancer cells may have the properties of CSCs**!

What is cancer stem cell (CSC)?

- **Stem-like progenitor cells** or **CSCs** are a small part of that heterogeneous population that could originate from *normal or stem cell* mutations initiated by changes in the environment, by chronic inflammation, or by epithelial-to-mesenchymal transformation (EMT).

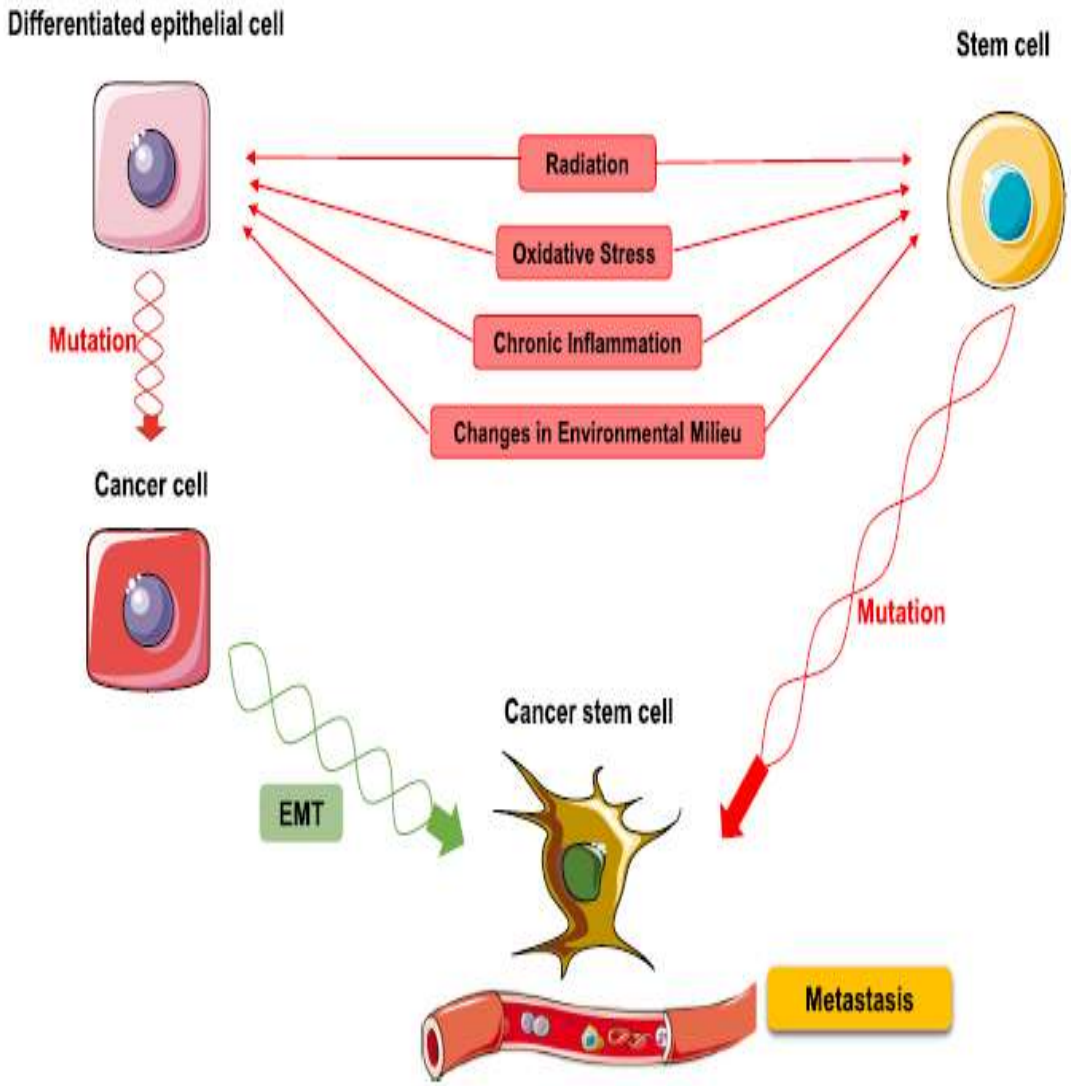
Role of EMT and MET in CSC establishment and progression

- EMT is a phenomenon observed during normal embryonic development and tissue repair and is characterized by epithelial cell losing their cell polarity, cell to cell adhesion, and gaining migratory properties and eventually transforming into MSCs.

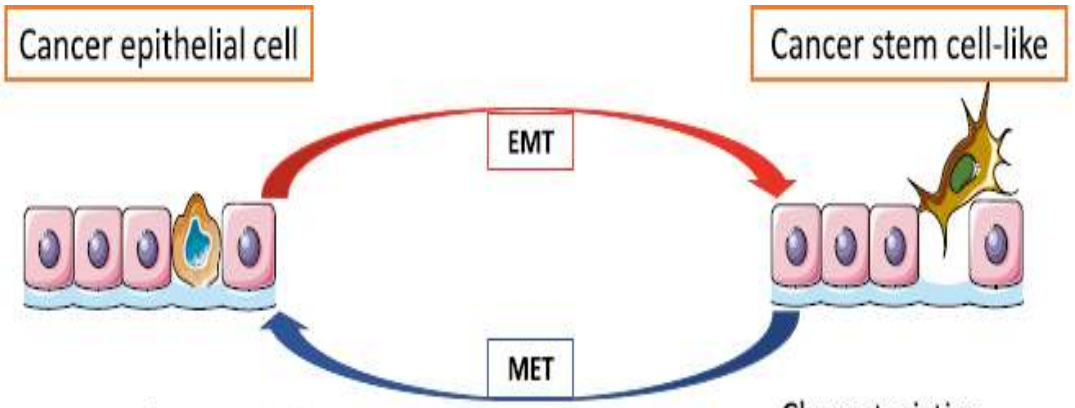
- EMT and its opposite process called mesenchymal-toepithelial transition (MET) are important processes that occur during embryonic development. Besides its role during embryonic development, it is known that EMT occurs in other physiological process such as wound healing, and in the development of organ fibrosis.

- EMT process has shown to be involved in cancer initiation and metastatic progression EMT contributes to cancer metastasis by facilitating local invasion, intravasation, transport, extravasation (by allowing cells to move to nearby blood vessels), and finally colonization.

- During cancer, EMT and MET show a dynamic relationship in which cells transiently undergo MET and in the next step undergo EMT to restart the metastatic process.



Rossi et al., 2020



Characteristics

- Non-invasive
- Cell Polarity
  - Apical
  - Basal
- Non-motile
- High expression of cell adhesion molecules
- E-cadherin expression

Characteristics

- Low (focal point) adhesion
- Invasive, migratory properties
- Stem cell-like behavior
- Highly mobile
- Decreased expression of cell adhesion molecules
- Decrease in epithelial marker E-cadherin

Rossi et al., 2020

III. Stem/Cancer Stem Cell Surface Markers/Isolation

Stem cell surface markers

- ESCs and SSCs can be distinguished according to specific intracellular and surface markers.

- There are some transcription factors that are commonly expressed in ESCs, the so-called core nuclear factors, like Oct-3/4, Sox2, KLF4, and Nanog.

- A wide range of cell surface markers characterizing ESCs have been reported, among the most common are the cluster of differentiation (CD) antigens surface proteins. CD antigens associated with pluripotency are CD9, CD24, and CD133. Additionally, ESCs express CD90, CD117.

- ESCs also express specific integrins that play a role in cell adhesion, signaling and cell migration. The most important are CD324 (E-cadherin) and CD29 (β1 integrin).

- Other proteins like TRA-1-60 and TRA-1-81, Frizzled5, and Cripto-1 are also characteristic of ESCs.

III. Stem/Cancer Stem Cell Surface Markers/Isolation

Cancer stem cell surface markers

- CSCs have been identified in several solid tumors based on the expression of certain CSC surface markers.

- Until now, CSCs have been identified by surface markers that are common between different cancer types:

  CD24, CD29, CD44 CD90, CD133,

  aldehyde dehydrogenase 1 (ALDH1), and

  epithelial-specific antigen (ESA).

- Depending on the type of tissue from which they originate, they can express a variety of markers for each type of CSCs. Most importantly, the expression of these markers can be used for specific therapeutic targeting of CSCs.

Tumor type	Markers
Leukemia	**CD34$^+$** [191, 196–198]; **CD38$^-$** [191, 197, 198]; **CD47$^+$** [191]; CCL-1 [191]; **CD96$^+$** [36, 191]; **CD90$^-$** [198]; **CD117$^-$** [198]; **CD133$^+$** [199]; **CD123$^+$** [191, 198]
Breast	**CD34$^+$** [200]; **CD24^{low}** [36, 48, 191, 197]; **ALDH1$^+$** [198]; **CD29$^+$** [201]; **Bmi-1$^+$** [202]; **CD133$^+$** [48]; **ESA$^+$** [191, 198]; **CD59$^+$** [36]
Pancreatic	**ESA$^+$** [48, 191]; **CD24$^+$** [48, 191, 198]; **CD44$^+$** [48, 191, 198]; **CD133$^+$** [36, 48, 198]
Lung	**CD44$^+$** [48, 191]; **CD133$^+$** [48, 191, 198, 203]; **CD59$^+$** [36]; **CD56$^+$** [203]
Liver	**ESA$^+$** [191]; **CD133$^+$** [48, 191, 198]; **CD90$^+$** [191, 198]; **CD44$^+$** [48, 191]; **CD176$^+$** [48]
Gastric	**CD133$^+$** [204]; **CD54$^+$** [48]; **CD44$^+$** [48, 191]
Colorectal	**ESA$^+$** [191]; **CD133$^+$** [36, 48, 191, 198, 205]; **CD166$^+$** [48, 191, 198]; **CD44$^+$** [48, 191, 198]; **CD24$^+$** [48, 191, 198]
Prostate	**Integrin α2β1** [48]; **CD44$^+$** [48, 198]; **CD133$^+$** [48, 198]; **Bmi-1** [206]
Melanoma	**CD34$^+$/CD31$^+$** [200]; **CD20$^+$** [198]; **CD44$^+$** [207]
Ovarian	**CD44$^+$** [48, 197, 208]; **CD117$^+$** [48, 197, 208]; **CD133$^+$** [48, 208]; **CD24$^+$** [48]

Rossi et al., 2020

III. Stem/Cancer Stem Cell Surface Markers/Isolation

Isolation

- SSCs are thought to be tissue-specific, which means that they give rise to progeny that correspond to their tissue of origin.
- SSCs can be found in adult tissues like the intestine, skin, muscle, blood, and nervous system.
- The two clinically utilized SSCs are HSCs and MSCs. HSCs are derived from the mesoderm and can be found in bone marrow and umbilical cord blood, and they give rise to blood cells during hematopoiesis and can be used in hematopoietic cell transplantation.
- MSCs are progenitor cells that give rise to cells representing different mesenchymal lineages and can be found in virtually any tissue but especially adipose, bone marrow, umbilical cord, and possibly in the human testis.
- The minimal criteria for MSCs are that they should be plastic-adherent in standard culture conditions, expression of CD73 (SH3), CD90, and CD150 (SH2) and lack of expression of CD11b, CD14, CD19, CD34, CD45, and HLA-DR molecules and be able to differentiate into chondrocytes, osteoblasts and adipocytes in vitro.

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To be continued…☺