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Escuela de Ciencias Biológicas e Ingeniería

TÍTULO:

Factors influencing the differentiation of embryonic stem cells in cancer cells

Trabajo de integración curricular presentado como requisito para la obtención del título de Ingeniería Biomédica

Autor:

Cevallos Chicaiza Josselyn Alejandra

Tutor:

Ph.D. Nelson Santiago Vispo

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Dedicatoria

Con mucho cariño,

Quiero dedicar este trabajo de investigación a mi madre, por ser siempre mi principal fuente de motivación y admiración. Por cuidarme siempre y por esperar de mí siempre lo mejor. Porque hasta el final ella creyó en mí.

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Josselyn Alejandra Cevallos Chicaiza

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Factors influencing the differentiation of embryonic stem cells into cancer cells

Abstract

Nowadays, Stem cells are a well-known topic, mainly because of their significant advantages and discoveries. Especially in embryonic stem cells, even though this type of stem cell brings several ethical problems, these are the most versatile in several fields. These cells are known for their self-renewal and proliferation properties; however, these two characteristics are also common in cancer cells. Stem cells have a relatively long lifetime than normal cells, making them suitable for genetic mutations. Moreover, when there are mutations, even if these mutations are small, stem cells may lose control of their growth or their characteristics mentioned above, such as self-renewal and proliferation, which most likely leads them to become cancer cells. The origin of cancer stem cells (CSCs) is still unknown, but several hypotheses suggest that these CSCs are the leading promoters of cancer metastases. This derivation in cancer stem cells may be due to several factors such as mutations mentioned above, genomic instability, and microenvironment, which will be mentioned throughout this work. Besides, we will study these cells' main characteristics: self-renewal and proliferation as two factors that influence CSCs on a large scale. This, to focus on improving processes that use stem cells as the primary source for further advances.

Key words: Stem cell, cancer stem cell, cancer, self-renewal, microenvironment

Factores que influyen en la diferenciación de células madre en células cancerosas

Resumen

Hoy en día, las células madre son un tema muy conocido, principalmente por sus importantes ventajas y descubrimientos. Especialmente las células madre embrionarias, aunque este tipo de célula trae varios problemas éticos, estas son las más versátiles en varios campos. Estas células son conocidas por sus propiedades de auto-renovación y proliferación; sin embargo, estas dos características también son comunes en las células cancerosas. Las células madre tienen una vida útil más larga que las células normales, lo que las hace adecuadas para mutaciones genéticas. Además, cuando existen mutaciones, incluso si estas mutaciones son pequeñas, las células madre pueden perder el control de su crecimiento o de las características mencionadas anteriormente, como la auto-renovación y la proliferación, lo que muy probablemente las lleve a convertirse en células cancerosas. El origen de las células madre cancerosas (CSC) aún se desconoce, pero varias hipótesis sugieren que estas CSC son los principales promotores de las metástasis del cáncer. Esta derivación en las células madre cancerosas puede deberse a varios factores como las mutaciones mencionadas anteriormente, la inestabilidad genómica y el microambiente, que se mencionarán a lo largo de este trabajo. Además, estudiaremos las principales características de estas células: la auto-renovación y la proliferación como dos factores que influyen en las CSC a gran escala. Esto, para enfocarse en mejorar los procesos que utilizan células madre como fuente principal para futuros avances.

Palabras clave: Células madre, células madre cancerígenas, cáncer, auto-renovación, microambiente.

Index

Abbreviations

Stem Cells (SC)

Induced pluripotent stem cells (iPSC)

Multipotent Stem Cells (MSC)

Transforming growth factoralpha (TGF-a)

Epithelial growth factor (EGF)

Hematopoietic Stem Cell (HSC)

Embryonal Stem Cells (ESC)

Cancer Stem Cells (CSC)

Phosphatidylinositol (GPI)

Dipeptidil peptidasa-4 (DPP4)

Sonic Hedgehog signage (Shh)

Real-time quantitative reverse transcription-PCR (qRT-PCR)

Green fluorescent protein (GFP)

T-cell factor (LEF-1/TCF)

Familial adenomatous polyposis (FAP)

Indian hedgehog (Ihh)

World Health Organization (WHO)

Pan American Health Organization (PAHO)

Basal cell carcinoma (BCC)

Medulloblastoma (MB)

Chapter I

1. Introduction.

Currently, there is a well-known issue but at the same time a very feared issue called Cancer. This complex theme has years of research and different treatments, but they still cannot find a cure. So years later, they are still under research. Cancer is the abnormal growth of a cell, so the human body unfamiliar with this begins to have complications. Different factors can be the cause of cause cancer. Including lifestyle and diet, and others may be due to genetic factors, mutations, microenvironment factors, or carcinogenic agents. Moreover, in some cases, the origin of this Cancer is unknown. According to the World Health Organization in the United States, this disease is so common today; in 2018, 616,714 died from some cancer; this figure is exorbitant. But let's focus on closer data, in Ecuador the same year, 14,559 died from cancer. (1) Given that our population is much smaller than that of the United States, this figure remains exorbitant. Finding a solution to this disease or finding treatments to stop people from dying is a priority.

On the other hand, we have another issue just as controversial as a disease mentioned above, but this issue is the topic that gives hope. The famous stem cells, nowadays everyone has heard of them. Now there are creams, hair, and beauty products with stem cells obtained from different plants and have had cosmetic benefits. All these products are already in our daily life. Besides, they have brought many discoveries and significant advances in the scientific field, but it is still in research as it is such a versatile subject. But why are they so important? Its unique and different characteristics are the key, such as differentiation in any cell type and its ability to self-renew. (2) These stem cell characteristics fulfill many functions, such as the regeneration of damaged tissues and the replacement of dying cells, their incredible proliferation capacity, and many more. Being for all this so important in the treatment of various diseases.

However, these novel features are also characteristic of cancer stem cells. (3) And several studies suggested that these cancer stem cells are promoters of cancer or its progression. As mentioned earlier, mutations can cause cancer because cells' lifespan is relatively short; mutations are supposed to accumulate in stem cells because their lifespan

is much longer. For a cell to have a malignant transformation, sometimes it requires a buildup of mutations; sometimes, a small mutation can cause the cell to lose its growth control or its self-renewal or proliferation characteristics. (3) This probably leads these cells to become cancer cells.

Despite all the years since the cancer stem cells (CSCs) were discovered, their exact origin is unknown even today, but several hypotheses suggest that these CSCs are the leading promoters of cancer, its metastasis, or its progression. Malignant transformation of stem cells into cancer stem cells may be due to several factors such as the mutations mentioned above, the microenvironment of each cell, oncogenes involved in self-renewal, and genetic instability. (4) These factors are what we're going to talk about in this research work. Also, we will study the main characteristics of these cells: self-renewal and proliferation as two factors influencing large-scale CSCs. (5) What are the most important markers in identifying CSCs and several important signaling pathways in several CSC processes. All this focuses on improving the processes that use stem cells as the main source for future advances.

Chapter II

2. Problem statement

In the world, the second cause of death after cardiovascular disease is cancer. Approximately 1.4 million people died from this terrible disease in America. This figure is only from 2018, not counting the 3.8 million people diagnosed with this disease. (6) Cancer is one of the top 4 non-communicable diseases (NCDs); these have an average of approximately 41 million samples per year. (1) This death toll is very high, equivalent to 71% of deaths worldwide, which does not show that this remains a problem of global importance. Let's get into statistics closer to our environment. In our country Ecuador, in 2018 statistics, 14.559 died from cancer, and in the same year, 28.058 were diagnosed with the same disease. (1) In Ecuador, cancer with the highest mortality rate is stomach cancer, followed by pancreatic cancer. In countries close to our countries such as Colombia or Peru the numbers do not decrease. In the same year, deaths in these countries were 46,057 and 33,098, respectively. (1) All this statistical data continues to give us a very clear idea that this disease attacks and kills millions of people worldwide. Any scientific advancement or research is helping to something more significant so that a future this disease stops killing so many people.

Although studies and research are progressing, the population must also do their part so that this problem does not continue to move forward. If each person does not take action soon for their own life, it is estimated that by 2030 the number of people diagnosed with this disease will increase by 32%, and this is as a result of several causes that influence the creation and progression of cancer. (1)(6) The population's factors to lower this index are their lifestyle, feeding, physical activity, and even annual medical checkups as prevention. Ecuador is an underdeveloped country with high mortality rates due to the lack of health budgets for treatments, medicines, or medical equipment needed for the correct treatments. Even the lack of budgets is what prevents the proper development of scientific research. Adding poverty to all this is an important factor in NCDs. Since the most vulnerable, socially, and economically unstable people are the ones who get sick the most and die before they can even receive the right treatment. (1)(6)

Promoting scientific research is key to our country's development; every research containing valuable and essential information generates an aid for the future of many research and generates knowledge. This paper seeks to determine the factors that

influence a normal stem cell to have a malignant transformation that promotes cancer stem cells' development. Thanks to several research, it has come to the assumption that thanks to these own characteristics of the stem cells acquired in these cancer cells, they are responsible for promoting tumor growth and cancer progression, which is why it is the importance of this work.

3. Objectives.

3.1.General objective

Conduct Bibliographic research of the factors that influence stem cells' malignant transformation into cancer stem cells using scientific articles obtained from secure databases such as PubMed to help approach new cancer therapies.

3.2.Specific objectives

- List and describe all existing stem cell classes.
- Identify the most critical specific markers for CSC identification.
- List and describe common properties between stem cells and cancer stem cells.
- Find and describe factors that are related to malignant stem cell transformation.
- List and describe biomedical applications to fight or help to prevent cancer.

4. Methodology.

The kind of study to be carried out is a narrative bibliographic review. The aim of this research is the bibliographic search for articles or reviews that contain information of interest to our topic; this will consist of definitions, descriptions, characteristics, markers, signaling pathways, and factors that influence the development of cancer, as well as applications. In this paper, three processes are distinguished: bibliographic search, analysis of the information found, selection, and synthesis.

Bibliographic search, different databases used:

PubMed: National Center for Biotechnology Information (NCBI) is responsible for this search system. This database allows us access to bibliographic databases grouped by the NLM: Medline, Genback, PreMedline, and Complete Genoma. Medline is one of the most important bases, where you can find several fields such as medicine, nursing, public health, oncology, and most sciences and research. This base currently consists of more than 15 million bibliographic references.

We have performed the following search string for this database to specify the search and shorten the results. The search string used has been as follows: stem cells, cancer stem cells, cancer, therapy, self-renewal, microenvironment.

Google Scholar: This database is from Google and focuses on searches that contain scientific-academic information. Here we can find articles, books, theses, documents related to congresses, patents, and abstracts. Their information comes from university publishers, universities, professional associations, and other academic organizations.

For this database, we have performed the following search string: stem cells, cancer stem cells, and cancer, therapy, self-renewal, microenvironment.

Other resources used include publications from international institutions such as the World Health Organization (WHO), Pan American Health Organization (PAHO).

Analysis and synthesis of information

This process was carried out with the collection of collected found; each document was reviewed one by one, selecting only the essential information, taking care that each data is valid, and eliminating the unnecessary accumulation of contradictions.

Inclusion/exclusion criteria:

- Study population: Unrestricted.
- Language restriction: Articles were filtered with English as the main language and Spanish as a second language. Articles with language in German, Polish, Chinese, and Japanese were discarded.
- Time Restriction: No time restrictions.
- Type of Study: Unrestricted.
- Keywords were included in the study title, summary, text, or keywords.

Vancouver is used as a publication standard for the bibliography.

Chapter III

5. STEM CELL (SC)

Stem cells (SC), also called precursor cells, are cells that can form new specialized cells that make up the different tissues (Figure 1) and organs of the human body or, in other words, these cells have self-renewal properties. They are like a type of master cells. (2) This feature may allow our organs and tissues to repair damaged tissue or renew cells from different tissues. These SCs can be found in different places in our body, they can be classified into embryonic stem cells, obtained from the embryo, and adult stem cells, found in adult tissues or umbilical cord stem cells. On the other hand, depending on the potential to generate different cells, SCs can be classified as unipotent, multipotent, pluripotent, or induced pluripotential (iPSC) totipotent stem cells. (7) It has different classifications to explain them in the first case according to their place of origin and the second case to evaluate their differentiation potential. Each type of SC will be explained in detail later. (2)(7)

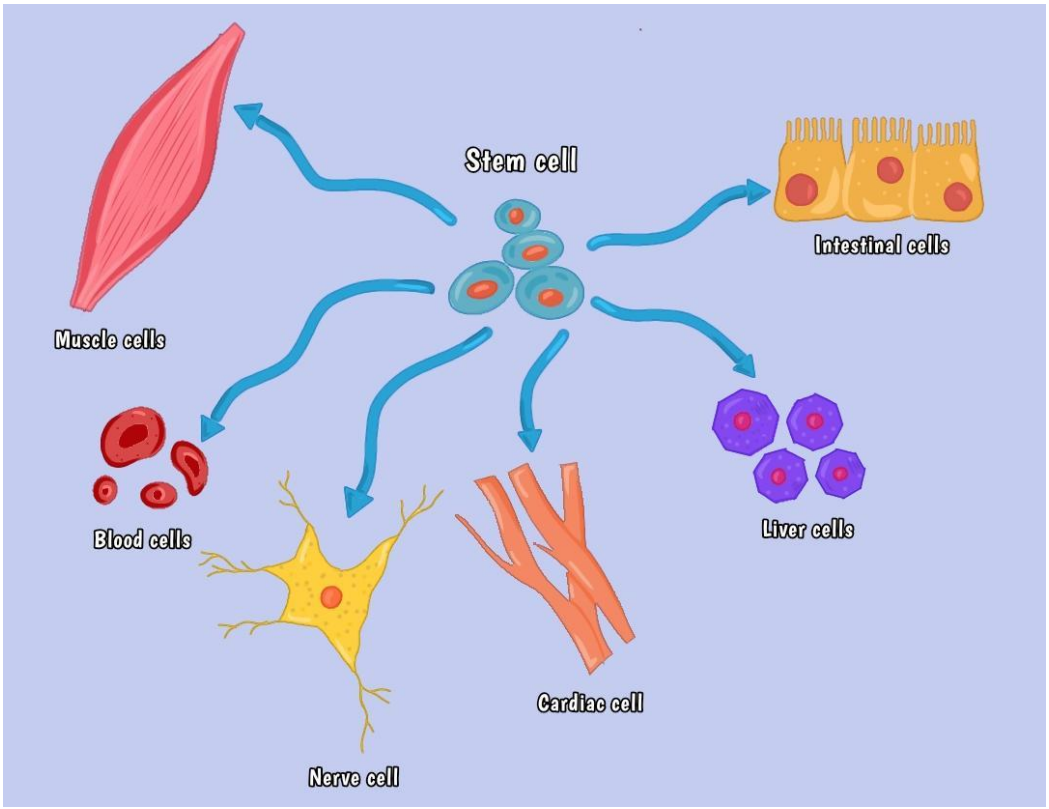


Figure 1: Different types of tissues in which a stem cell can be differentiated

Stem Cells, according to their potential for differentiation, can be classified as follows:

5.1. Totipotent Stem Cells:

This type of CS is the most interesting but the most complex. These cells in the right conditions can generate a complete embryo, which can be embryonic or extra-embryonic tissue (such as chorion, yolk sac, amnios, and allantoids). Put differently, they can be able to develop an entire individual. Hence its origin in the Latin Totus, which means complete. (2) These cells have essential molecular characteristics that define them as totipotent cells. We will mention the defining characteristic is the global epigenetic reprogramming, which has been studied in mice and humans in early pre-implantation embryos. (8) In this reprogramming, during the first cell divisions by establishing the internal cell mass, transient DNA demethylation occurs here. This transitory DNA demethylation is observed; here, most CpGs (DNA region where a guanine nucleotide follows a cytosine nucleotide) in stage two to eight hypomethylated when still in a totipotent state. (8)

5.2. Pluripotent Stem Cells:

This type of SC can give origin to progenitors to self-renew or differentiate in any of the three germ layers as they are: Mesoderm, ectoderm, and endoderm. These three layers are vital since they are later differentiated into different tissues and organs. (2) On the other hand, these stem cells are created when the internal cell mass forms when the trophectoderm (lineage) is established. (8) Besides, for these SCs to maintain a state of in-differentiation, they depend on or have the help of feeder cells. Diaz et al. give an example of these feeder cells to the mouse's embryonic fibroblasts or an extracellular matrix such as Matrigel. (9)

On the other hand, there are also induced Pluripotent Stem Cells (iPSCs) in this class. These iPSC's were first created in humans in 2007, and the extraordinary thing about them is that they can be reprogramed to function as embryonic cells. iPSC has characteristics that are the same as those of embryonic SCs, such as telomerase activity

expression, express the same cell surface markers such as several genes. (10) This is why this type of stem cell is also often called embryonic stem cells. However, for induced pluripotent stem cells to be considered good or suitable for use, they must meet certain morphological aspects.

According to Bilic J and Izpisua J. For a cell line to be considered a functional iPSC, it must meet some basic criteria such as the following. The first criterion we are going to mention is the proliferation rate; the second is the ability to form teratomas. And the third criteria are reactive pluripotency genes, followed by silence transgenes that are used for reprogramming. They should contribute to embryonic tissues when injected into blastocysts. Finally, to demonstrate the pluripotency capacity of iPSC, it must form a complete animal by tetraploid supplementation. (11) The problem with performing these tests to check for clear and rigorous pluripotency lies in its difficulty in routinely performing it on several cell lines

However, with all these characteristics, iPSCs can help develop new drugs or improve them, and once they eliminate technical limitations, they can help apply transplants. (10)

5.3. Multipotent Stem Cells (MSC):

These cells can differentiate or self-renew into a single specific range of cell types. This type of SC is considered adult stem cells because they have a reduced or limited capacity to differentiate into one or more cell lines. (12) A clear example to explain this type of SC, according to V. Rodriguez, are stem cells that give rise to tissues derived exclusively from the endoderm, such as pancreatic or lung tissue. (2)

As I have just mentioned, this type of SC has limited differentiation capacity, although it can produce other cell lines within its lineage. To explain this, we can take the multipotent stem cells of the brain as an example; the multipotent stem cells of the brain; these SCs can generate glial stem cells or neuronal cells, even different hematopoietic cells. These hematopoietic cells can be differentiated in most blood cells. (12)

However, multipotent stem cells can differentiate within their lineage, but they cannot differentiate into more brain cells. (12) Bone marrow is one of the most critical

sources where we can find multipotent stem cells. We can mention two of the most critical bone marrow stromal cells and tiny embryonic-like stem cells known as VSELs. (2)(12) In the bone marrow, we can also find more cells such as hematopoietic stem cells and endothelial progenitor cells (12); however, they are not multipotent, but it is necessary to mention that the bone marrow is some source rich in stem cells. This type of SC has a long list of properties; according to Sobhani the main trophic properties are: First, to induce cell proliferation and angiogenesis, the trophic factor is the expression of growth factors chemokines. Transforming growth factor-alpha (TGF-A), epithelial growth factor (EGF), hepatocyte growth factor. These three are mitogenic proteins produced by MSC to improve the division of epithelial cells, fibroblasts, and endothelial cells. (12)

On the other hand, including all the properties that have just been mentioned, Multipotent stem cells have anti-apoptotic properties, these properties are still under investigation; however, several anti-apoptotic proteins present in multipotent stem cells have already been identified, such as the IGF-1 protein and also the IL-6 protein, these two are responsible for regulating Akt protein also known as protein kinase B. (12)

5.4. Unipotent Stem Cells:

This type of SC is unidirectional with its differentiation capacity. This means that they can only be differentiated into one cell type or along a single cell line which is the same. (2) That's why these stem cells have the lowest potential for differentiation compared to other types of stem cells. An example may be muscle stem cells, another example being skin stem cells. Skin cells are constantly renewing. (2) That is why, if we take a small piece of this skin that is undamaged and in good condition, we can use these cells to grow them until we generate more tissues that are useful to be transplanted into a patient who needs it, for example, in someone who has skin problems due to burns. (2)(12)

Now, stem cells have already been classified according to their differentiation capacity, there is also another classification according to the tissue where they can be obtained, here we find two classes embryonic stem cells and adult stem cells.

5.5. Embryonic stem cells

Embryonic stem cells can be obtained in the early stages of the embryo when the fertilized ovum is still a morula in its 3 to 5 days of life. After these 3 to 5 days of fertilization, the embryo cells have been divided into a new structure called a blastocyst consisting of about 500 cells. (13) The cells that form the blastocyst are responsible for giving rise to all types of tissues, cells, and organs of the individual that begins to form. Embryonic stem cells are found in the blastocyst's internal cell mass; they can divide continuously and then have the ability to differentiate themselves to form any tissue in the body. (14) As mentioned above, and in general, the function of most stem cells (SCs) can differentiate into any type of cell or tissue to aid in the regeneration or fix. (15) However, this type of stem cell has several ethical problems since, when obtained from embryos can be considered as interrupting a human life being for further research with embryonic stem cells; however, rules have already been raised for this issue. Without counting on the fact that there are fertilization clinics where these SCs can be obtained without harming anyone and with the consent of the people going to donate, the patient is always informed. (2)(13)

5.6. Adult Stem Cells.

This stem cells are found in as their name says adult tissue, although in a smaller number, such as in the bone marrow, nervous system, or even the pancreas. This type of SC has less differentiation capacity as they are often inclined to follow the cell line of the organ or tissue where it was removed. (2) After several studies, thanks to the plasticity of SC, these can be differentiated into different tissues. Within adult stem cells, we can find several types such as hematopoietic, mesenchymal, neural, epithelial stem cells, but they are the main ones that we will mention quickly. (16)

5.6.1. Hematopoietic stem cell (HCS).

Hematopoietic stem cell is mainly found in the bone marrow, although in a small amount, it would equal 1 out of every 10000 cells in the bone marrow, which is a percentage equivalent of 0.05% to 0.5%. (17) From this type of CS all blood cells are

derived or the whole spectrum of globules. Blood cells can be classified in two ways: lymphoid cells made up of T cells, B cells, and natural killer cells. On the other hand, we have the myeloid cells made up of granulocytes, monocytes, erythrocytes. (17) All of these blood cells have a relatively short lifetime. They can last for hours for granulocytes or last for up to weeks for red blood cells. (17) Now, understanding this, these blood cells are in constant and permanent production. As just mentioned, hematopoietic stem cells only follow one cell line, which is that they are usually known as multipotent stem cells.

After differentiating into progenitor cells, these hematopoietic stem cells lose their self-renew ability to result in mature blood cells. Once these mature blood cells are formed, they have a regulation in their progression according to the following stages; Mainly the commitment to a specific cell line, besides also due to this lineage restriction, its differentiation is also restricted by the same and to finish, the cell growth stops, and apoptosis occurs. (17) These stem cells have medical applications, such as the treatment of insufficiency bone marrow, which involves the infusion of identical allogeneic hematopoietic stem cells through a central venous pathway. (2) (17)

5.6.2. Mesenchymal stem cells (MSC).

Some know these types of stem cells as skeletal stem cells. Their primary source of getting is bone marrow, although they have also been identified in different tissues such as the pancreas, adipose tissue, liver, skeletal muscle, also has been identified in the blood of the umbilical cord, among others. After looking at the previous idea, the primary source is bone marrow; however, only 0.03% of cells in this area are MSC. (2)(18) These cells have some restrictions that limit their use; among them, we can mention their low growth rate, Besides that, when being samples taken directly from patients, it is necessary to consider the ages of donors without counting the fact that the risk can be high at the time of sampling. (18)

According to Arévalo et al. In 2006, 3 important criteria were proposed so that a mesenchymal stem cell can be defined as such; To be considered, mesenchymal cells must be adherents in the culture. When hematopoietic antigens CD34, CD45, macrophages, markers of monocytes, and B lymphocytes are absent, the cells should express the CD73, CD90, and CD105 antigens. Finally, mesenchymal stem cells should

be able to differentiate in vitro in both adipocytes, osteoblasts, and chondrocytes, and they should be under standard conditions of the culture. (18) These being the main characteristics that a mesenchymal stem cell must meet to be used as such.

5.6.3. Skin stem cells.

Skin stem cells have the same base characteristics as any stem cell, such as self-renewal and differentiation, only that this differentiation is within their cell line so that it would have limited or restricted differentiation. These skin stem cells' primary function is to rejuvenate skin cells through the tissues' homeostasis, which also regenerates the hair. Another essential function is the repair of the epidermis after a wound or injury. (19) The epidermis is vital on a large scale in the human body as it forms an entire protective layer along with its appendages, which protect humans from harmful microbes and prevent possible dehydration. Also, the self-renewal capacity of stem cells in the epidermis is pervasive; we can give us an example of a cell-based that has been wholly differentiated in four weeks and has gone to the surface of the skin. (19)

The source of these stem cells is in the adult hair follicle, the epidermis basal layer, and the sebaceous gland. To be more accurate, multipotent stem cells of the follicle and epidermal cells are found as their name says in the epidermis, whereas the melanocyte stem cells are found in the hair follicle region. These three sources of obtaining or parent populations express the proteins K5, K14, and p63. (19)

6. CANCER STEM CELL (CSC)

To start talking about this topic, we must define several terms that we will use throughout the article, such as:

Heterogeneity: heterogeneity is when their elements that compose it are distinguishable from each other and form, in turn, part of the same set, mixture, or group. Heterogeneous tumors are divided into spatial and temporal types depending on whether the non-uniform distribution of cancer cells is dispersed through and within sites of the disease or whether there is a variation of the cell within a certain period. (20)(21)

Cancer: cancer is caused by mutations or changes within the DNA of a specific cell that possesses a wide variety of individual genes; this may be due to genetic instability or even environmental factors. (3) As mentioned above, each cell has many individual genes; these are responsible for performing different functions, but we are currently growing and dividing. A mutation that affects any of these functions can promote a cell's malfunction and become a cancerous cell. (3)(22)

Tumorigenesis: The process of tumorigenesis occurs when normal cells acquire malignant properties. This process has several characteristics, such as de-differentiation and the reprogramming of somatic cells. (23) Continuing with the characteristics, this process shows an extremely quick proliferation, effectively evades apoptosis, plus there is the presence of metastasis and metabolism without regulation. All these features we just mentioned are a fundamental part of cancer. However, these malignant properties could be related to the different studies in which several somatic genetic mutations have been identified. (23)

6.1. Models on the origin of cancer.

Cancer is an issue that is still in constant study. Its origin is described in two models, the clonal model and the model of cancer stem cells, which we will explain below.

The first model that could describe Cancer's origin is called a hierarchical model or cancer stem cells theory. This theory proposes that a small population of stem cells is responsible for initiating the tumor, maintaining the tumor, and producing oncogenicity (promoting cancer progression). (24) All functions that we have mentioned above would occur due to the unique properties of stem cells, such as self-renewal and proliferation. This model could explain the heterogeneity present in neoplastic processes, the continued resistance to radiotherapies and chemotherapies in cancer treatments, where these cancer cells survive, re-appear, and promote tumor recurrence. (22) (4)

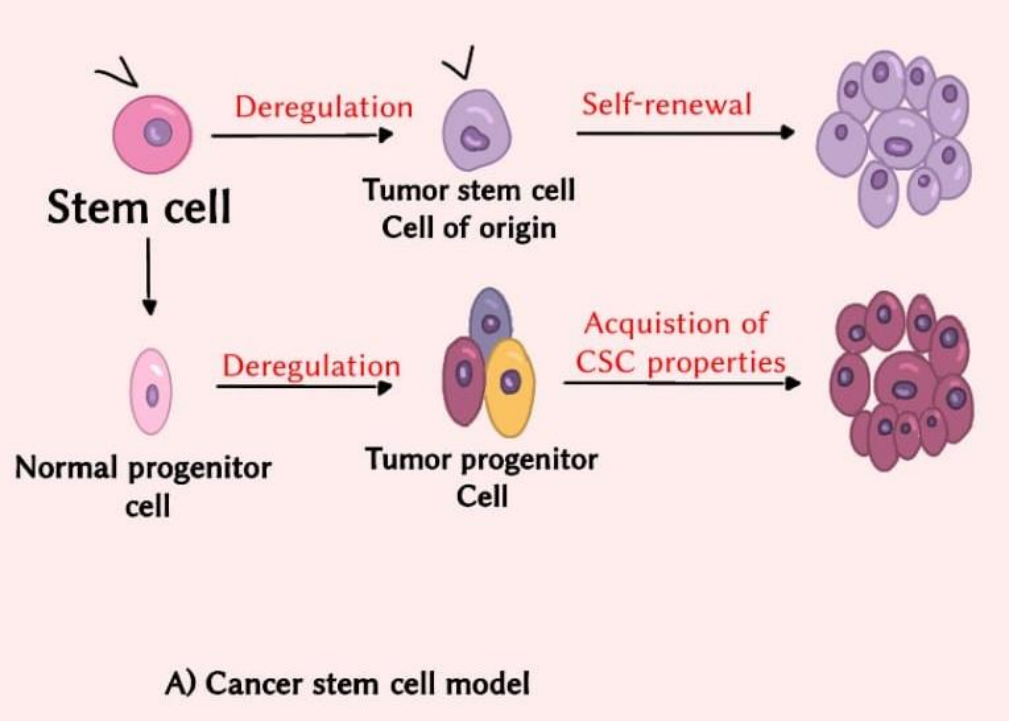


Figure 2: Model of Cancer Stem cell

In the case of the second model, called the clonal model or stochastic model. Nowel described this model in 1976, which proposes that any stem cell can present a mutation and acquire the potential to start the tumor through a division process that gets out of control what causes an accumulation of genetic alterations until it reaches a tumor level; this process would occur randomly. (4)(22)

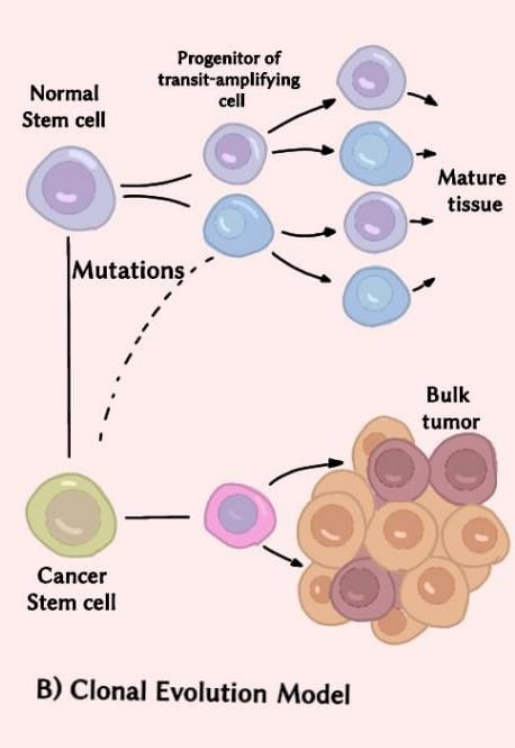


Figure 3: Clonal Evolution Model

6.2. Cancer stem cells (CMCs)

On the other hand, we have cancer stem cells; they are also known as cancer-initiating cells. These are neoplastic cells that, in contrast to SC, they have a potential for self-renewal that acts indefinitely. This means that it does not control its renewal capacity, which makes it oncogenic. (25) This type of CSCs cell can originate in different cells, both in normal stem cells, but they can also occur in differentiated cells but have acquired characteristics of a normal stem cell. For this type of cell to acquire its oncogenic capacity, it must possess several characteristics. Below we will mention the most important ones: We can start by saying that one characteristic is the accumulation of mutations throughout its life, (4) (25) these cells have an indefinite proliferation, another key feature is their resistance to apoptosis (cell death), they have abnormal growth and do not follow the indicated signaling of anti-growth, so we can say that they grow too fast and they extend to different organs, on the other hand, we also have another important feature that is the increase of cellular motility, in other words, what we know well as metastasis (spreading to different organs). (4)(26) And we must mainly consider the symmetrical division

responsible for self-renewal; in clearer words, it is responsible for creating daughter cells from the cell division of a stem cell. (4)(27)

Chapter IV

7. FORMING CSCs

7.1. How CSCs are extended or divided.

Carcinogenic stem cells, like cells in different tissues, have two forms of division; these can occur randomly or in response to environmental signals. (5) Underneath, we will explain each type of division:

The first division is symmetrical; all stem cells are divided into two, in a daughter cell and another stem cell. (28) When a stem cell needs to expand in number (grow) it uses the symmetrical division; a clear example of this division occurs in injuries or wounds during its development or its healing. (5) On the other hand, we have the second division, which is asymmetrical; in this case, the cells are based on the available space they have in the niche to reproduce; this means that it can be divided zero, one, or two times. (28) This division usually occurs at a steady-state; in this state, the asymmetrical divisions are the ones that allow a balance between stem cells, exactly in normal stem cells and differentiated stem cells. (5) In this division, we also have the intrinsically asymmetric divisions; these allow stem cells to regulate their self-renew by asymmetric segregation of different determinants that guide the daughter cell. An example described by Shenghui He, Daisuke Nakada, and Sean J. Morrison; *Drosophila neuroblasts divide asymmetrically by segregating atypical protein kinase C to daughters fated to remain as stem cells and Numb, Prospero, and Brat to daughters fated to differentiate.* (5)

However, for these cells' proper functioning, there is a balance between these two types of divisions in both symmetrical and asymmetrical to restrict or limit cancer progression. (5) Nevertheless, after tumorigenesis occurs, there is a change in divisions; one might say that there is an improvement in divisions. Which could lead to an expansion

of cancer stem cells that can trigger a more robust, more dangerous, and undifferentiated state of a carcinogenic tumor. (28) This occurs because cells near the niche that are compromised send signals to stem cells to maintain their properties and restrict their differentiation. (28) We can conclude that both CSCs and normal stem cells can vary and achieve self-renewal capacity depending on the environmental signals they are exposed to, including may become equally tumorigenic either of the two equally. (28)

7.2. Stem Cells for Malignant Transformation

Numerous research proposes that stem cells play an essential role in the development of cancer. First, stem cells could develop into cancer cells by acquiring mutations. However, we must be taken into account that both progenitor cells and mature cells have a functional life that is limited, which means they live little. That is why the chances that all mutations can be stored in them are meager. For this reason, it follows that these mutations are stored most likely in stem cells, and because of their unique properties, they can transform into cancer cells. (26) The second point we will address is self-renewal and regulation of expansion, these two properties of stem cells are under strict regulation. However, if the expansion process does not have this strict regulation on stem cells, it could create cancer. As a third point, cancer tends to have the ability to self-renew, so it is suggested that they come from stem cells that own this property. (26) Proliferation and self-renewal, as mentioned above, are key characteristics of cancer stem cells. For this reason, these two characteristics must be out of strict genetic regulation to be considered carcinogenic. Therefore, as these characteristics are unique to stem cells, it is suggested that stem cells are the ones that undergo malignant transformation. Unlike progenitor cells that do not possess these characteristics and would be more complicated, it is impossible to become malignant. Then, we can say that cancer stem cells need the deregulation of these two properties, proliferation, and self-renewal. (26)

7.3. Progenitor Cells for Malignant Transformation

As mentioned in the previous section, the most accurate hypothesis in terms of studies and evidence is that stem cells are the ones that are recurrently affected by mutations, and after these mutations, malignant transformations are likely to occur. These

would result in cancer cells originating in normal stem cells. There could also be a small group of malignant transformations from progenitor cells. Besides, according to Clarke, it is likely that progenitor cells, possibly arising from mutated stem cells, may be transformed by subsequent genetic events that confer immortality and/or self-renewal potential to these normally non-self-renewing cells. (26) This means that a progenitor cell is derived from a stem cell that already accumulates mutations. After several events, it can become malignant after its division and formation into a progenitor cell by different genetic processes; all of these circumstances gave self-renewal properties to a cell that does not possess this property. Several studies in both humans and mice support this hypothesis, where stem cells harbor the accumulation of oncogenic mutations that will then lead to malignant transformation. (26)

8. MARKERS used for Identification of CSCs

The most commonly used means of identifying cancer stem cells is based on cell surface markers' expression; these markers also enrich or isolate cancer stem cells. (29) These cells are complicated to identify as they are hidden in tumors, which further complicates their elimination. (30) And although many markers have already been identified, some are still in research. According to con Kim W Y Ryu C *most of the current CSC surface markers are derived from known normal embryonic or adult stem cell surface markers*. (29) The similarities in cell surface markers suggest that these cancer stem cells can originate mainly from normal stem cells through different genetic alterations or mutations. (29)

Markers can be found due to the density of tumor tissues due to related genes' expression and the signature of proteins present in CSCs. This occurs because the expression of the surface markers of the cancer stem cells is not the same as those of other cells; if these markers are found in other tissues or an unrelated organ gives us an advantage, it could help us identify the type of cells in which they are present. (28)(31)

Acute myeloid leukemia (AML) was the key to identifying cancer stem cells (CSC). Two cell surface markers that were CD34+ and CD38- were identified with AML. This study was conducted in mice; they transplanted the subset of cells of the CD34+ and

CD38- in mice with diabetes and combined immunodeficiency (NOD/SCID). (32) This process led the mouse to restart the same leukemia. These results led us to discover the existence of cancer stem cells in liquid tumors through surface markers. (29)

The markers most found in cancer stem cells are CD24, CD26, CD44, CD133, CD166, CD326, and Ep/CAM; these are examples of specific markers on CSC. (28) On the other hand, several of the same markers that have just been mentioned are used in solid tumors to identify cancer stem cells, but we also found CD34. (28) On the other hand, we also find the CD29 markers known as Integrin β1, in addition to those already mentioned CD44, CD133, CD166 CD326, these markers have a primary function, measuring the adhesion of cells in the niche. (28) The ALDH1 marker is an enzyme called aldehyde dehydrogenase 1; this marker is responsible for the oxidation of aldehydes, but it is also related to metastasis and tumorigenesis in cancer stem cells. (28)

Many more markers have been found on different cell surfaces. However, many are still under research, as mentioned above. Still, let's summarize below the surface markers found in cancer stem cells, with their properties and expressions in Table1. As it was described above, there are some predominant markers in cancer stem cells' study. For that reason, below, we will explain a little each of them.

Let's start with CD24; this marker is a glycosyl phosphatidylinositol (GPI)-linked sialoprotein with a molecular mass in a range of 35-60 kDa. (29)(33) This marker is rarely expressed in normal tissue. CD24 is mostly expressed in undifferentiated ESCs, but also in human neural lineages. Because this marker is expressed in several types of cancer, it is considered a marker of cancer stem cells; we can also say that this marker in combination with CD44. This combination may help in the identification of breast cancer stem cells. (29)(34)

The CD26 marker is a seine DPP4, which is expressed in a diverse group of cells. This marker is rarely expressed in normal tissue, but it is expressed in pluripotent stem cells and hematopoietic stem cells. (35) Exactly it is expressed in large proportion in cells of kidney tissue, small intestine. As for detecting cancer stem cells, the CD26 marker is mainly involved in leukemic stem cells and colorectal CSCs. (29)

The CD44 marker is one of the markers most studied in different cancer stem cells. This marker is a hyaluronic acid receptor, and it has a family that encompasses many isoforms. These isoforms are expressed by cutting and the alternative coupling of the pre-mRNA. (29)(36) This marker is expressed primarily in hematopoietic stem cells and cells derived from adipose tissue. The CD44 has been expressed in many normal tissues, but in the CSCs field, its function is still a little limited. However, much of it has been used combined with other putative markers to isolate CSCs in solid tumors. (37) The standard isoform of this CD44s marker is a glycoprotein that it has as its primary function, cell adhesion, and cell migration, but it has an essential role in binding to hyaluronic acid in extracellular matrices. (29) On the other hand, different studies show that several markers such as CD44v9 have emerged as an important marker in different solid tumors. Also, more of this variant has been used as markers of cancer stem cells in different cancers. (28)(29)

On the other hand, we have the marker CD133, also known as preminin-1, a glycosylated protein. This marker is one of the most studied when it comes to solid cancers. Its function is to organize the cell membrane's topology; this marker is usually expressed in embryonic and neural stem cells. It is also said that this protein is downregulated by undifferentiated embryonic stem cells. The CD133 marker has been identified in several CSC populations such as in breast, lung, brain, liver, pancreatic, ovarian, colon cancer. Marker CD166 has been identified mainly on the surface of proliferating cells; besides they have been identified in epithelial cells that are already differentiated in different tissues. (29)

The next marker we're going to talk about is the CD166. This marker is a member of the immunoglobulin family, and it is a membrane glycoprotein specifically of group I. This glycoprotein has been expressed on the surface of epithelial cells. However, it also expressed although weakly in undifferentiated embryonic stem cells. (36) This marker has been found mainly in intestinal stem cells and stromal stem cells. This marker is usually used mainly in detection colorectal cancer stem cells. Also, in different study's, this surface marker of malignant stem cells has been identified in an inert manner for lung cancer. (29)

EpCAM is also known as CD326 or as an epithelial cell adhesion molecule. This marker is a transmembrane glycoprotein responsible for the mediation of adhesion between cells in the same way, independent of Ca2+ in epithelial cells. (38) The CD326 is also found on a large scale in adenocarcinomas, and it expressed in some normal epithelial cells and tissues. Studies show that this glycoprotein is involved in tumor metastasis and so on in cancer stem cells. In undifferentiated embryonic stem cells, CD326 is also used as a marker. (28)(29)

The last marker we're going to talk about is CD34, which was first found in hematopoietic progenitor cells. Its expression is scarce in normal tissues, except, for example, progenitor cells and hematopoietic stem cells, as mentioned above. (32) These markers were involved in discovering cancer stem cells in a study of acute myeloid leukemia (AML), where CD34+ and CD38 – identified leukemia stem cells. (29)

The following table lists cancer stem cells' surface markers that they have been most found and have more information. Besides, there is their expression in embryonic stem cells (ESC), adult stem cells, and their expression in normal tissue cells. (33)

Table 1.

CSC surface markers expressed on ESCs, adult stem cells or in normal cells						
CSC surface marker	**Function / Origin**	**Expression in CSCs**	**Expression in ESC**	**Expression in ASC**	**Expression in normal tissue/cells**	**Ref.**
CD24	B cell proliferation	Breast, gastric, pancreas	Yes	Intestinal	Infrequent (B lymphoid, neural)	(29) (33) (34)
CD26 (DPP-4)	Dipeptidyl peptidase iv, FDA-approved target	Colorectal, leukemia	Yes	Hematopoietic	Infrequent (intestine, kidney, male, female tissues)	(29) (35)
CD133 (AC133)	Marker for hematopoietic stem cells.	Breast, prostate, colon, glioma, liver, lung, ovary	Yes	Hematopoietic Neural Prostate	Infrequent (proliferative cell)	(29) (39) (40)
CD326 (EpCAM)	Cell adhesion, signal transduction	Colon, pancreas, liver	Yes	No	Infrequent (epithelial cell)	(29) (41) (38)

CD90 (Thy-1)	Signal transduction/ cell adhesion	Brain, liver	Yes	Mesenchymal, cardiac	Infrequent (T-cell, neuron)	(29) (42)
CD49f (Integrin α6)	Cell adhesion	Glioma	Yes	Hematopoietic	Infrequent (rectum, urinary bladder)	(29) (43)
CD146 (MCAM)	Melanoma cell adhesion molecule	Rhabdoid tumor, sarcoma	Yes	Mesenchymal	Infrequent (endothelial, ganglion cell)	(29) (34) (44)
CD10 (Neprilysin)	Metallo-endopeptidase, FDA-approved target	Breast, head and neck	Yes	Mesenchymal	Infrequent (glandular cells)	(29) (34) (45)
CD117	Receptor for stem cell factor (FDA-approved)	Ovary	Yes	Mesenchymal Cardiac	Infrequent (myeloid)	(29) (34) (46)
SSEA3	ESC marker	Breast, teratocarcinoma	Yes	Mesenchymal	Infrequent	(29) (47)
SSEA4	ESC marker	Teratocarcinoma, breast	Yes	Mesenchymal, cardiac	Infrequent	(29) (48)
SSEA1	Mouse ESC marker	Teratocarcinoma, renal, lung	Yes (mouse)	Cardiac	Infrequent	(29) (49) (50)
Cripto-1 (TDGF1)	Self-renewal/survival in esc	Breast, colon, lung	Yes	-	Infrequent (pancreas, hippocampus)	(29) (51) (52)
Notch2	Signal transduction	Pancreas, lung	Yes	Neural	Infrequent (intestine)	(29) (53) (54)
ABCG2	ATP-binding cassette transporter	Lung, breast, brain	Yes	Hematopoietic Muscle Neural	Infrequent	(29) (55)
CD29 (Integrin β1)	Cell adhesion, FDA-approved target	Breast. Colon	Yes	Mesenchymal	Ubiquitously	(29) (34) (56)
CD44 variants	Hyaluronic acid receptor, FDA-approved target	HNSCC, breast, colon, liver, ovarian, pancreas, gastric	No	Hematopoietic Adipose Mesenchymal	Lymphatic tissues and epithelial	(29) (36) (37)

CD166 (ALCAM)	Cell-cell/cell-matrix interaction	Colorectal, lung	Yes (weak)	Adipose, intestine	Many epithelial cells	(29) (36) (34)
CD9	Cell adhesion	Leukemia	Yes	Adipose-derived mesenchyma	Many tissues (except liver, gall bladder)	(29) (46) (57)
ABCB5	Transporter of ABC	Melanoma	-	Limbal	Normal tissues	(29) (60)
CD123	Receptor for IL-3	Leukemia	-	No	Normal tissues	(29) (58) (59)
Notch3	Signal transduction	Pancreas, lung	-	Neural	Many tissues	(29) (53) (54)
CD34	Cell adhesion	Leukemia, squamous cell carcinoma	No	Hematopoietic	Infrequent (lymphoid)	(29) (32) (61)
CD271	Nerve growth factor receptor	Melanoma, head and neck	No	Mesenchymal	Infrequent (neural crest)	(29) (61) (62)
CD13 (Alanine aminopeptidase)	Kidney disease marker	Liver	No	Mesenchymal	Infrequent	(29) (63) (64)
CD54	FDA-approved. Cell adhesion.	Gastric	No	Mesenchymal	Infrequent g	(29) (69) (70)
CD56 (NCAM)	Cell adhesion	Lung	No	Mesenchymal	Infrequent (lymphoid)	(29) (65)
CD105	Coreceptor for TGF-β	Renal	No	Mesenchymal	Rare (endothelial)	(29) (66) (67)
CD114	Colony stimulating factor 3 receptor	Neuroblastoma	No	Neural crest, BM-derived precursors	Infrequent (brain, skin, placenta, BM, heart)	(29) (68)
CD55 (DAF)	Inhibitor of complement	Breast	-	-	Infrequent (lymphoid)	(29) (71)
CD20	FDA-approved. B cell lineage	Melanoma	No	No	Infrequent	(29) (72) (73)
TIM-3 (HAVCR2)	Immune checkpoint receptor	Leukemia	-	-	Infrequent (lymphoid)	(29) (79)

CD96	T cell-specific receptor	Leukemia	-	No	Infrequent	(29) (74)
LGR5	Cell adhesion	Intestinal, colorectal	No	Intestinal, kidney, stomach, hair follicle	Infrequent (brain, intestine)	(29) (75) (76)
CXCR1, 2	Chemokine receptor	Breast, pancreas	-	Mesenchymal	Infrequent	(29) (77) (78)

Identifying many cancer stem cells (CSCs) through cell surface markers has prompted much research. With the theory that if these cells are removed, perhaps it could eliminate whole tumors. This assumption is based on the fact that cancer stem cells are the only ones responsible for the self-renewal process, and without them, this process could not continue. According to Won-Tae Kim & Chun Jeih Ryu: Cancer stem cells were identified on their cell surface based on their molecules. The development of specific antibodies and immunotoxins that focus on the surface molecules of CSC to eradicate them selectively is under investigation. (29) This issue is still under investigation, but it is certainly a significant advance in cancer treatment.

9. Features and properties that share so many normal stem cells and cancer stem cells

• Self-renewal:

This property is responsible for the creation of new stem cells. This is one of the properties of great interest today as its use would be very beneficial in several more scientific aspects; however, it is still in research. Self-renewal is often confused with proliferation, as the two processes have an essential dependence on cell division; nevertheless, they are not the same. (5)(80) Let us explain the proliferation of quickly form because it will be explained further below. Proliferation incorporates cell division of several cells such as stem cells and progenitor cells, but generally of any type. Now, yes, let's start talking about self-renewal; these processes are more specific, this one needs at least one of their daughter cells to be the same, or it has a similar development to that of the stem cell. (80) This means that at least one of their daughter cells must be a stem cell. To illustrate this, we can consider hematopoietic stem cells (most mammalian cells);

for them, this self-renewal means the division of their cells but maintaining their multipotency of them. (5)(28)

Self-renewal process in embryonic stem cells (ESC).

To initiate this process in ESCs, a positive feedback loop is formed through a transcription regulatory network Oct4-Sox2-Nanog to regulate the expression of genes responsible for promoting differentiation negatively.(81) To help suppress the expression of genes associated with this differentiation that we're talking about, they use Polycomb family proteins (PcGs). Besides, to suppress differentiation, the MARK pathway signaling inhibition uses leukemia inhibitor factor (LIF) signaling, along with bone morphogenetic protein (BMP). (5) The autocrine fibroblast growth factor (FGF) is responsible for the activation of this MAPK pathway signaling. In conclusion, we can conclude that embryonic stem cells have their self-renewal process by inhibiting differentiation. (5)(82) When cells are found in crops with conditions where there no occurs differentiation encouragements, self-renewal may occur, nevertheless, in the case of embryonic stem cells. They can spontaneously differentiate as the FGF factor accumulates and as the LIF/BMP factor is depleted when it reaches high densities in the culture. (5)(82)

- **Proliferation:**

This property is responsible for the indefinite division that owns each SC. This is one of the most controversial characteristics because while controlled, it is very beneficial to humans, but if this control is lost, it can cause unmeasured growth or expansion that, together with mutations, are responsible for the tumors continuing to grow and multiply. (83)(84)

Cell proliferation is the process in which a cell grows to divide to create two daughter cells. That is why this process is the one in charge of the increases the number of cells. Put differently, this method is responsible for the growth of the tissue. An interesting fact of this process is that protein p53 is a negative regulator in the cell cycle.

This would increase cell proliferation due to deregulation caused by gene alterations induced by this protein. (83)

Chapter V

10. Factors influencing the formation of cancer stem cells

In the previous chapter, we saw that self-renewal plays a vital role in cancer development, but we also saw that this property is key or typical of both normal stem cells and cancer stem cells. A failure or alteration in any of the auto-renewal processes could be the promoter of some type of cancer, so we have selected this as a critical factor in stem cells' malignant transformation. Secondly, we have the microenvironment of stem cells or, in other words, the niche. These cells have a coating that sends signals for correct proliferation, and some alterations in these signals or in the niche, in general, could also promote alterations, which could produce accelerated growth, this would lead us to select this as the second factor for malignant stem cell transformation.

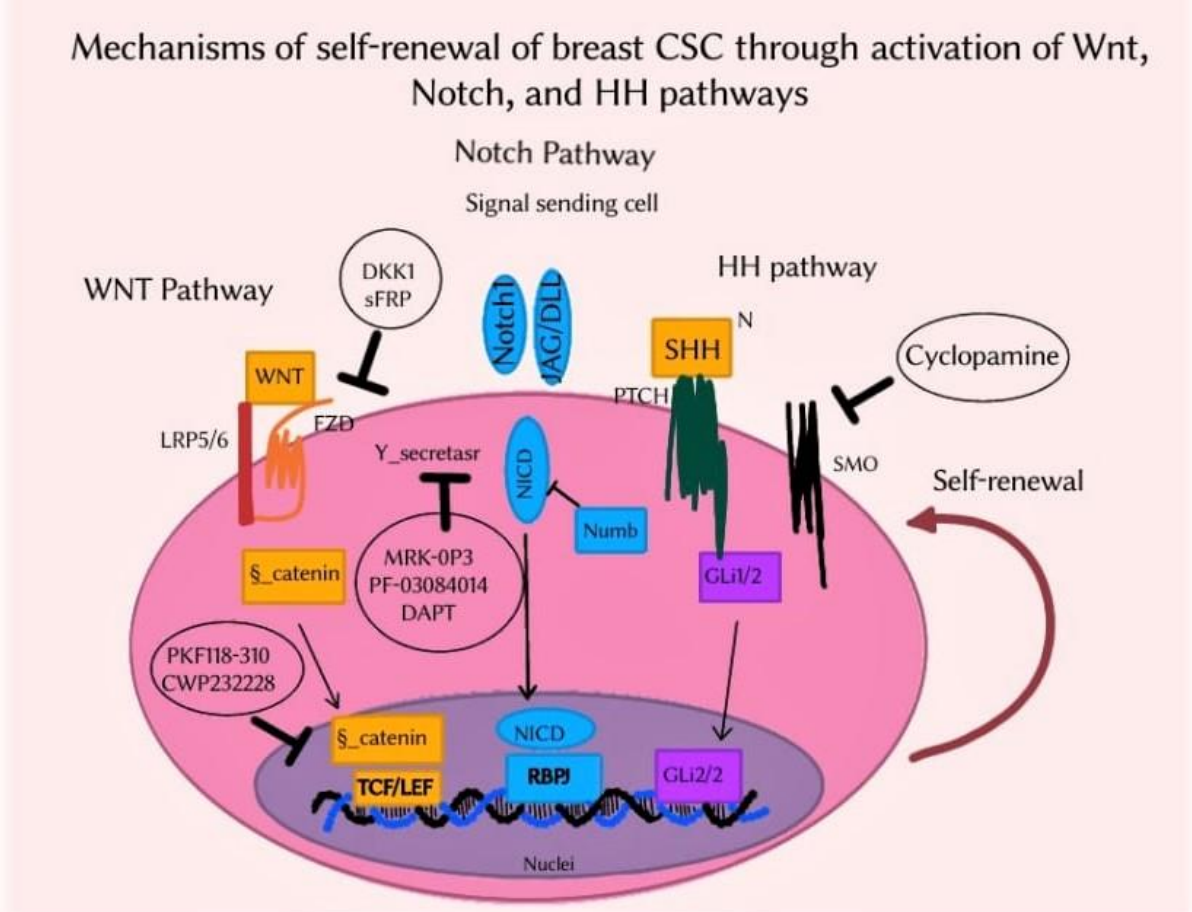
10.1. Multiple oncogenes involved in self-renewal

As already explained, self-renewal is a critical property in both stem cells and cancer stem cells. In contrast to stem cells, cancer stem cells lose their control, precisely their homeostatic control, thus losing the strict genetic regulation that stably keeps stem cell reserves. (28) This causes a steady expansion in the number of stem cells. Also, this results in an unmeasured proliferation, it leading to tumor growth. Next, let us expound on some of the factors that influence the operation of self-renewal. (4)

Let us explain some examples or evidence that the same factors that influence self-renewal in stem cells are the same ones that influence the malignant transformation of the same cells. Let us initiate with Sonic Hedgehog (Shh) signaling that showed a relationship with self-renewal regulation. When a population of cells stimulated with Sonic Hedgehog in vitro showed an increase in self-renewal ability, thanks to this stimulation. (26) In Figure 4, we can see the functioning of the Hedgehog signaling pathway. Another clear example is the Hox family; the expression of HoxB4 is also involved in stem cell

functions. (59) And the last example we can give is the bmi-1 gene, which in conjunction with c-myc can induce lymphoma in mice, but on the contrary, we can say that the same gene is necessary for the maintenance of leukemic cells but also in hematopoietic stem cells. (26)

Several pathways, Wnt/ β-catenin, and Notch have been implicated in mouse cancer and human cancer. But these same pathways have also been involved in the self-renewal property of normal and carcinogenic stem cells. (85) In Figure 4 we can see the functioning of the Wnt/ β-catenin and Notch signaling pathway.



In Figure 4. Functioning of the Hedgehog, Wnt *and Notch signaling pathway in the self-renewal.*

Wnt/β-catenin signaling has been an essential factor in self-renewal and the malignant transformation of normal stem cells. It is also very useful to consider that Wnt / β-catenin is interrelated with several more signaling pathways such as Notch, Hedgehog,

mTOR; these pathways have as their primary objective the coordination of the development of the different organs or in some cases, these signals maintain homeostasis in specific tissues. (85) One of the first appearances of the Wnt pathway in cancer was discovering that some murine breast tumors from the insertion of the mouse breast tumor virus into the Wnt-1 gene prompted an expression without locus regulation. (85)

This activation pathway has an essential dependence on the cytoplasmic concentration of β-catenin. This protein is usually in low concentrations due to a degradation process. The Wnt ligand activates the signaling pathway and inhibits β-catenin phosphorylation, resulting in its degradation by ubiquitin-proteasome. (28) The increase of this protein allows its entry into the nucleus; here, this protein activates the transcription of a select group of genes. And several protein products of these genes are involved in the processes of cell division, morphogenesis, and embryonic development. (85)

Different interactions with this signaling pathway have been seen in different studies. Inhibition of Wnt/ β-catenin through ectopic expression of axin promotes both in vivo and in vitro cell proliferation inhibition in stem cells. In other studies, the Wnt/β-catenin pathway is involved in the self-renewal of progenitor cells or stem cells in different tissues. Studies have shown that cells' proliferative activity is directly related to the β-catenin level of a specific keratinocyte. (26)

The absence of the β-catenin protein in the nucleus, the target genes of Wnt are usually inhibited by several factors such as lymphoid stimulating factor (LSF) and T cell factor (LSF-1/TCF); these are joined to a co-receptor protein. The Wnt ligand produces the inhibition of the degradation of the protein β-catenin. (86) This results in the accumulation of cytoplasmic β-catenin, which after its translocation in the nucleus, formed an LSF-1/TCF complex. Besides, it will adopt a co-activator function because it induces the transcription of the Wnt pathway's target genes. Several target genes of the Wnt pathway are related to cell proliferation and growth; these genes include c-Jum, c-Myc, PPARD, FOSL1, CCND1, among others. (85) As we can see, this signaling pathway performs several essential functions, so we can say that some defect or alteration in the regulation of this route is Wnt signaling; it could lead to the development of diseases or be carcinogenic. (85)

Alterations from the Wnt pathway cause several diseases. This may be because early discoveries of the Wnt genes categorized them as a proto-oncogene family. The Wnt/β-catenin pathway is one of the 4 known pathways of the Wnt family; Wnt/β-catenin will been identified as one of the principal responsible for cellular alterations that could subsequently lead to some form of cancer. (87) Thanks to several studies, several genes that regulate this signaling pathway are altered in human cancers. (85) Some examples of the Wnt/β-catenin pathway genes present in many types of cancer are: The APC gene is present in colorectal cancer or in family adenomatous polyposis FAP in these diseases, the APC gene causes loss of function. (85) (86) The AXINA 1 gene is found in hepatocellular carcinoma, colorectal cancer, or esophageal squamous cell carcinoma, and this gene also causes loss of function. (85)(86) The β-catenin gene is found in several diseases such as hepatocellular carcinoma, prostate cancer, melanoma, ovarian cancer, this gene produces genetic mutations. (85,86) And the last gene we're going to mention is FRPs, and it's found in pancreatic cancer, sporadic colorectal cancer, or renal cell carcinoma, and the gene can cause methylation. (85,86)

Furthermore, of these diseases associated with some alteration in this signaling pathway's genes, some of these genes have been found that after they have acquired a targeted mutation, they can promote cancer in animal models. (85) Several studies have concluded that a common factor in all the cases mentioned above is modifying or altering the Wnt/ β-catenin signaling pathway's target genes. (85) And the last point to mention about this signaling pathway is that several studies have also found a relationship of this pathway to stem cell survival and to proliferation alterations. (85)

Moreover, to Wnt/β-catenin signaling, we have the Notch signaling pathway, which plays different roles in various cell development events; these can range from proliferation, self-renewal, growth, migration, apoptosis, among others. (88) Notch's family was first discovered in Drosophila; in this fruit fly, it acted as responsible for ectoderm and neurogenesis specification. (26,88) In vivo studies, the activation of Notch in a hematopoietic stem cell culture temporarily increases parents' number. This suggests that Notch is involved in maintaining progenitor cells' multipotentiality and in the self-renewal of hematopoietic stem cells. Besides, the Notch pathway was found in cancer in

cancer by identifying an int-3 mouse oncogene that ended up being a truncated Notch-4. But it should be mentioned that the Notch pathway is more complex to understand in human cancer. Notch's family has been implicated in some cancers of epithelial origin. In leukemia, instead, Notch activation has been involved by chromosomal translocation. (53) Another family member of Notch's signaling pathways, specifical inhibition of Notch-1, may induce cell death or, in other words, apoptosis in leukemia cell lines. In contrast, overexpression of the same gene results in a stop in the growth of a cell line of small cells in lung cancers. (26,88)

These signaling pathways are related to the proper development of self-renewal. This property is present in both normal stem cells and cancer stem cells, so it could be assumed that some alteration in the development of normal stem cells could be involved in the malignant transformation of the same cells into carcinogenic.

10.1.1. Clinical Trial.

In the following study, they performed an in vitro model and a xenograft mouse model to examine the function of Hedgehog signaling and Bmi-1 signaling in the process of self-renewal; it focuses on normal human breast stem cells and malignant stem cells. It was shown that Hedgehog signaling components such as PTCH1, Gli1, and Gli2 are expressed in normal human breast stem cells. (89) These stem cells are cultivated as mammospheres; these mammospheres are spherical colonies in suspension cultures from human breast stem cells isolated from reduction mammoplasties. It is essential to mention that these non-adherent mammospheres have a high level of stem cells and mammary progenitors but maintain their self-renewal and differentiation properties in multiple lineages. (89)

Furthermore, these Hedgehog signaling genes are negatively regulated when stem cells or progenitor cells are induced to differentiate themselves. By activating Hedgehog signaling mediated by the polycomb Bmi-1 gene, the initiating cells of the mammospheres are increased, also the size of the mammospheres increases. (89) Conversely, if Hedgehog signaling is inhibited, the opposite occurs, and these same effects are reduced. On the other hand, the Gli2 gene of the same signaling, if

overexpressed in the mammospheres' initiating cells, can result in ductal hyperplasia. Another favorable result of this study was that Hedgehog signaling is activated in cancer stem cells by $CD44^{+}$ $CD24^{-/low}$ markers. (89)

We can conclude from this essay something important. This study supports the hypothesis of a cancer stem cell model where the Hedgehog pathway and Bmi-1 pathway interaction actively participate in essential functions in the self-renewal of normal stem cells and tumorigenic. (89)

10.1.1.1. Description of the procedure and materials used in the clinical trial.

1. Dissociation of breast tissue and mammosphere culture.

- For creating the mammospheres, between 100 and 200g of normal breast tissue were ground and dissociated, individual cells were subsequently cultured in suspension. (89) This tissue is derived from reducing mammoplasties.
- After the primary mammospheres were dissociated, they were grown in suspension to produce mammospheres. After that, these mammospheres were created, or these cells reach 85% confluence. It was isolated in RNA using a mini-kit from RNeasy, used for Real-time quantitative reverse transcription-PCR (qRT-PCR). (89)

2. Treatment of Mammospheres.

- In six-wells (ultra-low) junction plates, individual cells of epithelial organoids were sown. (89,90)
- The biological active used were: Unmodified amino-terminal recombinant human Sonic hedgehog and mouse Indian Hedgehog (Shh), cyclopamine (Ihh). (89)
- Correct inhibition was obtained with 3 ug / mL of Shh or 300 nmol / L cyclopamine. For cyclopamine it was used as a negative control to tomatidine. (89–91)

- The mammospheres were collected on days 1.3,5, or 7. These mammospheres that they were already collected were used for RNA and qRT-PCR extraction. (89–91)

Notes: They were tested with different concentrations of Shh until they reached the correct stimulation. Mammospheres treated for 7 days were used for in vitro self-renewal assays. (89)

3. Immunostaining.

- In collagen-coated plates, single-celled suspensions were sown for 7 days. It was performed to evaluate the composition of colony lineage. (89)
- During 20 minutes, the cells were attached to plates in a methanol solution at -20ºC and were dyed with the kits. (89)
- The primary antibodies used in solutions were cytokeratin 18 (in epithelial cells) and cytokeratin 14 (in myoepitational cells). (89)
- AEC and 3.3'-diaminobenzidine were used as substrates for peroxidase and nitroblue tetrazolium / 5-bromo-4-chloro-3-indolyl phosphate. For alkaline phosphatase. (89)

4**. Virus production, cell culture, and infection.**

- Vector only (SIN-IP-EGFP), Gli1 (SIN-GLI1-EGFP), Gli2 (SIN-GLI2-EGFP). (89)
- Retroviruses were created by transfection of 293 cells (stable). This process was carried out to infect the individual breast cells isolated from the primary mammospheres. (89)
- To generate lentiviruses expressing the Bim-1 and green fluorescent protein (GFP), a highly efficient lentivirus expression system called pLentiLox 3.7 was used. (89)

5. RNA constructions (Small interfering)

- Three hBmi-1 siRNA oligonucleotides were used to confirm the deletion of BMI-1 expression, this elimination occurred in primary breast epithelial cells. (89)
- To be attempted into a vector of lentivirus LentiLox 3.7, siRNA sequences were converted into smaller forks now called shRNA. (89)
- GFP was used as a marker in cells infected with lentivitus to indicate that cells express small hairpins (shRNA) for human Bmi-1 (hBmi-1). (89)

Notes: In this study less than 90% of cells were infected with control (GFP alone) or siRNA lentiviruses (hBmi-1-siRNA1-GFP, hBmi-1-siRNA2-GFP, and hBmi- 1-siRNA3-GFP).

6. Implantation of mammospheres in the purified fat pads of NOD-SCID mice.

- Following a protocol, three-week-old mice (NOD-SCID) were anesthetized (only in females), this was done with an intraperitoneal injection or i.p. injection The injection was 0.2 ml ketamine xylazine. For every 20 g of the mouse, 0.02 ml of a 300 mg solution of Ketamine combined with 20 mg xylacine was used in a volume of 4 ml. (33,89)
- Four posterior or inguinal mammary glands were cleaned. Subsequently, human breast fibroblasts immortalized were humanized with 2,5x10^5 non-irradiated telomerase and with the same amount of irradiated fibroblasts. (33,89)
- A 60-day estrogen-release plate was placed on the back neck of the mouse. This was done using a trocar and by mixing 400 mammospheres previously prepared with 2,5x10^5 normal human breast fibroblasts. They were then resuspended in 10 ul with a relationship of 1:1 Matrigel, in a serum called F-12 of Ham that was at 5%. And finally, they were injected into each of the fat pads. (33,89)

Notes: i.p injections are commonly used in small rodents. Each implantation experiment tube had 5 repetitions using different mammospheres of each patient. Three mice were used for each patient sample. (89)

7. Preparation of sections of breast fat pads.

- In approximately 8 weeks after implantation, the fat pads were removed and fixed for one hour in a Carnoy's solution. Then they proceeded to dye them overnight with carmine alum. (89)
- To continue the degreasing process, it was made with graded ethanol, and subsequently, the tissue was clarified with 5ml xylene in approximately 1 hour. (89)
- To complete this section, the tissue was paraffin-embedded and sectioned for H&E staining. (89)

8. Preparation of single-cell suspensions of tumor cells, flow cytometry, and xenografts.

- Transfer of human breast tumors to NOD-SCID mice. The tumors were first cut into small pieces and subsequently crushed. The obtained pieces were washed with serum-free HBSS. All of this was done before implantation. After this, a small cut of 2 mm was made in the mouse's abdomen using a trocar; 1 or 2 tumor pieces were implanted in the fat pad region. Then they were sutured, and the same pieces were removed 5 days later. (33)
- After 1 to 2 months in which the tumor has grown, the tumors were eliminated, and individual cells were obtained through collagenases' digestion. These cells were used for various processes. One part of these cells was used to classify the population of $H2K^{d-}$ $CD44^{+}$ $CD24^{-/low}$ $Lineage^{-}$ with the population $H2K^{d-}$ $CD44^{+}$ $CD24^{+}$ $Lineage^{-}$, this process was performed through flow cytometry. (33,89)
- The last step in this section was to extract RNA from each of these two populations to quantify gene expression using real-time RT-PCR. (33,89)

Notes: The samples of human breast tumors were received for implantation approximately 1 hour after surgeries to fresh.
The insertion region was just below the nipple on both sides of the chest in the fat pads. (89)

9. Statistical analysis.

- The results were calculated using statistical software called Minitab. They used one-way ANOVA. (89)
- $P < 0.05$ was considered statistically significant. (89)

10.1.1.2. Analysis of the results of the clinical Trail.

Several genes in the Hedgehog signaling pathway are expressed on a large scale in stem cells and breast progenitor cells. Table 2 describes the process and materials with which this experiment was developed. When grown under non-adherent conditions to primary human breast cells that were isolated from reduction mammoplasties, it was obtained that the vast majority of these cells suffered anoikis which is a type of programmed cell death. However, a small portion of cells (approximately 4 out of 1000 cells) can form spherical colonies that we call the mammospheres. Through the retroviral marking, they could show that the mammospheres can have the ability to dissociate, and it can switch to serial clonal density. This will occur with the second and subsequent

generation of mammospheres produced by individual cells, it maintaining a constant number of them after several generations. (89)

Differentiation.

To evaluate the differentiation potential, these cells were placed on collagen substrates at a clonal density. This study suggests that the mammospheres are formed by a small portion of stem cells responsible for creating more mammospheres, and they are also responsible for creating more progenitor cells in charge of differentiation in multilineage. However, they are not able to form spheres. Cells bound to collagen substrates are the ones that induce irreversible differentiation of these cells. Breast-derived cells were used in a suspension culture against breast-derived cells in the same way, but in this case, grown on a collagen substrate. This is to compare the expression of genes from the Hedgehog signaling pathway in breast stem cells against the breast cells, but in this other case, differentiated. (89)

The results showed that Ihh is hedgehog ligands mostly expressed in breast epithelial cells, and their expression is approximately 9 times higher in stem cells in mammospheres compared to differentiated cells that were cultured in collagen substrate. Hedgehog PTCH receptors are expressed approximately 4 times more in stem cells/breast progenitors than in differentiated cells. In contrast, the SMO receptor is expressed three times more in differentiated stem cells than in stem/progenitor cells. Similarly, with the Gli1 and Gli2 transcription factors. In stem cells/breast progenitors, it is expressed approximately 25 times more than in differentiated cells (Gli-1). And the Gli2 factor is expressed 6 times more in differentiated cells than in stem cells/breast progenitors. All of these results tell us that the Hedgehog signaling pathway acts favorably on stem cells/ breast progenitors, and the contrary, during differentiation, it is regulated negatively.(89)

Self-renewal

In this case, we examined the effects of Sonic Hedgehog (SHH) and its inhibitor cyclopamine on the formation of both primary and secondary mammospheres. The first result, we obtained a 57% increase in primary mammospheres using 3 ug/mL Shh and a 62% increase in mammospheres. By contrast, cyclopamine reduced the number of

mammospheres by 51% and 45% of primary mammospheres. The efficacy of Sonic Hedgehog was demonstrated by inhibition of it with cyclopamine. The degree of reversal of the cells depended on the concentrations used in both cyclopamine and Shh; this suggests that a low level of concentration, the Smoothened inhibition, does not occur ultimately. (89)

In the secondary mammospheres, they had an even more significant impact with Hedgehog signaling. These secondary mammospheres were formed from primary mammospheres that were treated with Shh. Similarly, the use of cyclopamine reduces the effect of SHH, the study verified it. Secondary mammospheres from primary mammospheres were reduced by 54%, compared with control results. (89)

However, to show that Hedgehog stimulates self-renewal, they were based on the potential for differentiation of the cells already treated with Hedgehog's linking. If this signaling pathway acted on primitive cells, then stimulating this pathway would also increase the number of cells capable of differentiating themselves. Therefore, mammospheres-derived cells were placed in collagen plates at a clonal density. Cytokeratin 14 was used as the myoepithelial cell marker, and cytokeratin 18 was used as a marker for epithelial cells. The results obtained by increasing Shh were an increase of 3.5 in the number of cells, whereas cyclopamine decreased the number of cells by 1,8. (89) This showed that Hedgehog activation increased the number of undifferentiated cells. Ihh and Shh had the same effects on the production of progenitors and on the formation of mammospheres. It was also experienced by increasing 3ug/mL of Shh or 300nmol/mL of cyclopamine not in the mammospheres but added to the breast epithelial cells that were previously grown on a collagen substrate. However, no visible effect was obtained on cell proliferation. This suggests that the Hedgehog pathway affects larger-scale proliferation in undifferentiated cells. (89)

On the other hand, we also have the Hedgehog signaling pathway's transcription factors, which are Gli1 and Gli2. To check if Gli's transcription factors are involved in self-renewal. Retroviral vectors containing the Gli1 and Gli2 factors were used to infect mammospheres initiating cells. To determine what effects these transcription factors have on the formation of mammospheres. (89) A retroviral expression system was used, and

overexpression of Gli1 and Gli2 was found to stimulate the production of mammospheres in breast epithelial cells in primary suspension cultures instead of controls. This production increase 49% with Gli1 and 66% with Gli2. There was also a 77% increase with Gli1 and 100% with Gli2 in the number of cells per mammosphere. All of this shows us that gli1 and Gli2 transcription factors influence Hedgehog activation. (89)

Bmi-1 in Self-renewal

The Bmi-1 gene was recently linked to the regulation of self-renewal. In studies, we found that Bmi-1 mRNA levels have an increase of 3.5 in mammospheres, which does not occur in undifferentiated breast cells. It is assumed that this gene would be acting downstream in Hedgehog signaling. To verify this, we look for the effect that Hedgehog activation has on the expression of the Bmi-1 gene. The results showed us that activating this pathway signaled with Shh increased the expression of Bmi-1 6 times more in mammospheres. However, this effect was blocked by the cyclopamine inhibitor that is specific to the Hedgehog signaling pathway. Similarly, Gli overexpression showed a 6-time increase in Bmi-1 in mammospheres compared to controls. These results suggest that Bmi-1 gene can be positively regulated in stem cells/ human breast progenitor cells through Hedgehog signaling. (89)

We are now focusing on self-renewal to see what effect the Bmi-1 gene has on this property. Lentiviral vectors (contain Bmi-1) were used to infect mammospheres initiating cells. Here we find that overexpression of the Bmi-1 gene stimulates mammospheres formation by 80% and increases the number of cells per mammospheres by 67% compared to cells that express GFP uninfected controls. To better corroborate this, another test was performed. A lentiviruses vector containing siRNA (labeled such GFP) was administered to down-regulate the expression of the Bmi-1 gene. (89)

Bmi-1 expression was reduced by the presence of two different siRNA lentiviruses. In mRNA, it produced an 80% reduction, and protein levels produced a 70% reduction. Thus, downregulation of Bmi-1 gene expression reduced the formation of primary mammospheres by 80% and reduced secondary mammospheres by 70%. In terms of the mammospheres' size, this downregulation was reduced by 60% in primary mammospheres and 70% in secondary mammospheres. So we can see that Hedgehog's

activation visibly reduced the formation of primary and secondary mammospheres through the down-regulation of Bmi-1. This study can conclude that the Hedgehog signaling pathway acts on stem cells/breast progenitors when mediated by the Bmi-1 gene. The experiment contains more parts; however, we only explain the parts of interest in our research. (89)

We can conclude that self-renewal was evaluated by cells derived from the mammospheres to form new mammospheres containing cells with Multipotent characteristics. And the differentiation potential was evaluated by growing cells in a collagen-coated substrate in the presence of serum. Besides, Bmi-1 is expressed in large numbers in undifferentiated breast cells, Bmi-1 increases its expression by activating Hedgehog signaling. (89) Self-renewal is influenced on a large scale by Bmi-1 overexpression, similarly the proliferation of breast stem cells. This was proven by increasing the number and sizes of breast cells in vitro. On the other hand, the down-regulation of Bmi-1 nullifies the Hedgehog signaling effects on creating in vitro mammospheres. All this suggested that the Bmi-1 is the one that acts on Hedgehog's signaling in self-renewal. Finally, both Hedgehog signaling and Bmi-1 act on the generation or progress of in vitro breast carcinomas or, in some cases, in transgenic models. (89)

10.2. Microenvironment involved in malignant transformation

A microenvironment is another factor we will study in the malignant transformation of stem cells into cancer stem cells. The microenvironment is also known as niche or paracrine signaling. This niche surrounds stem cells and seems to play an essential role in their regulation. (22) Niche interacts or is influenced by several factors such as autocrine signaling, in addition to stimuli from stromal fibroblasts, immune cells, endothelial cells, and extracellular matrix. There are also stimuli or physicochemical factors such as oxygen, tissue pH, or nutrient supply. (25) The regulation of several stem cell processes is due to the interaction between all the factors mentioned. Furthermore, niche generates extrinsic factors responsible for controlling proliferation, growth, number of cells, and they are also responsible for determining the destination to which stem cells will target. (92) Studies have shown several signaling pathways responsible for development processes, such as Hedgehog, Wnt, Notch, and fibroblast growth factors.

These pathways perform many functions in the regulation of self-renewal, and also they play essential roles in regulating the fate of the lineage in a diverse group of systems. (92)

The microenvironment keeps stem cells in a state of inactivity by providing signals that do not allow proliferation or cell growth to occur; in other words, these signals inhibit these two factors so that the stem cell remains in a state of inactivity. (92) For stem cells to begin their division and proliferation cycle, they need a signal that stimulates them to activate; this dynamic signal comes from the niche that surrounds them. For example, with bromodeoxyuridine, stem cells can retain this nucleotide for an extended period. (93)

Moreover, a correct balance between these proliferation and anti-proliferation signals is of great importance in regulating stem cells, specifically in homeostatic regulation. This process is essential because it allows stem cells to renew themselves but at the same time support the process of tissue regeneration that is also in process.(93) (94) However if any genetic mutation occurs, it can lead to independence in stem cells, which could lead to resistance to anti-growth or anti-proliferation signals. (95) This would lead to significant problems as the cells would have uncontrolled proliferation and subsequently possible tumorigenesis. (93) In figure 5 it can be appreciated a diagram showing this phenomenon. The functioning of the niche can be seen under normal conditions and in carcinogenic conditions.

10.2.1. Microenvironment in CSCs

In different studies, it has been disclosed that cancer stem cells reside in a vascular niche. (25) This niche promotes the properties of self-renewal and growth thanks to the signals it receives. These vascular endothelial cells have been identified as a critical resource in the niche, but they are specific to CSCs. It is suggested that these stem cells are located in the perivascular region of the niche. (25)

As mentioned above, the niche is influenced by several factors and the microenvironment in normal stem cells; autocrine signals also influence the microenvironment in CSCs or stimuli from fibroblasts, so are physicochemical factors such as oxygen or tissue pH. (96)

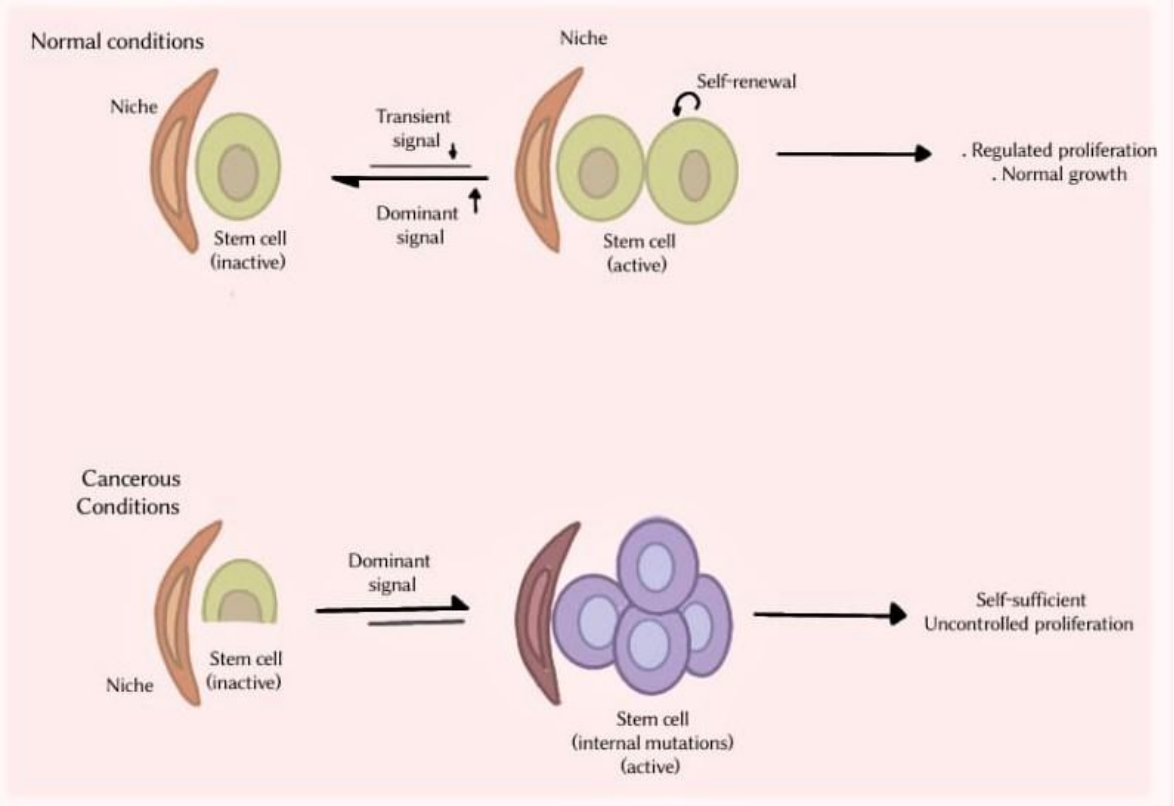


Figure 5. Niche under normal conditions and in carcinogenic conditions.

10.2.2. Hypoxia in the niche

The hypoxic niche is essential as it protects CSCs from chemotherapy and even radiation. (25) Solid tumors usually have regions subjected to hypoxia temporarily or transitory; this is due to incomplete and somewhat disorderly vascularity. The restriction in the supply of both nutrients and oxygen is caused for all of these. As a result, CSCs follow anaerobic glycolysis that produces higher lactic acid and lower pH. This process favors CSCs in the activation of proteases, stimulation of angiogenesis, among others that promote their progression and metastasis. (24)(95)

Hypoxia-inducible factors (HIFs) are part of the expression by hypoxia-induced CSCs. HIF genes are the leading promoters of angiogenesis by introducing vascular endothelial growth factor A (VEGF-A). VEGF-A production stimulates tumor vascularization when in hypoxia conditions. This VEGF-A is produced by endothelial cells and CSCs. Moreover, in conjunction with CSCs, cancer-associated fibroblasts are

responsible for producing CXC motif chemokine 12 (CXCL 12) in the same way to promote angiogenesis. (96)(97) Meanwhile, macrophages associated with tumors become angiogenic. This occurs through its response to the macrophage colony-stimulating factor (M-CSF), secreted by tumor cells. This results in the production of VEGF-A and the suppression of the expression of anti-angiogenic factors. (24)(92)

Reactive oxygen species (ROS) are abundant in the microenvironment because of their high metabolic activity. They increase when the oxygen concentration decreases. ROS are responsible for stimulating cell survival and participate in the induction of the epithelial-mesenchymal transition. These two processes that we have just mentioned occur through signaling the transforming growth factor-beta (TGF-β). (93) Hypoxia is also responsible for inhibiting cell proliferation by regulating low c-Myc expression. Furthermore, the signals of TGF-β and Wnt promote the state of undifferentiating. All this favors the properties of self-renewal and the generation of differentiated progeny. (24,97)

As has just been seen, hypoxia leads to a loss of control in regulating the microenvironment. Simultaneously, this deregulation promotes cancer growth and promotes the remodeling of the extracellular matrix that favors metastasis and promotes the vascularization process that facilitates cancer progression and suppresses the antitumor function. Finally, by inducing cell inactivity, it contributes to resistance to therapy. (24)

Next, there are some studies on regulating microenvironment signals to show the importance of microenvironment signals in stem cell control.

The skin stem cells are found in the hair follicle in its bulge area. In these cells, Wnt signaling plays the role of promoting and expanding these cells. However, Wnt inhibitors such as Dkk, sFRP, and Wif are also present in these cells' niche. (93)(96) Intestinal stem cells (ISCs) are found between the proliferating progenitor cells and the differentiated Paneth cells. In these cells, Wnt signaling promotes the proliferation of crypt cells. Nevertheless, Wnt inhibitors such as sFRP5 are also present. On the other hand, BMP signals and the transforming growth factor-β (TGF- β) are responsible for

providing signals that promote proliferation inhibition. To inhibit the proliferation and activation of stem cells in the same way. (93,95)

The Wnt signaling pathway is activated by binding with the Frizzled receiver, and together they send a signal to inhibit a hostile complex. This negative complex consists of glycogen synthase kinase-3β and adenomatous polyposis coli (APC). These two are responsible for phosphorylation and then degradation of β-catenin. When activated the Wnt abnormally produces a continuous signal of proliferation that results in a build-up of β-catenin and subsequently results in APC development, bone marrow leukemia, and tumors in the skin follicle. (93)

In a mouse model, the BMPR1a (it is a mediator of BMP2 /BMP4 signaling) is conditionally inactive. This inactivation resulted in expanding stem cells in the hair follicle, bone marrow, and intestinal. This, in turn, resulted in hair follicle tumors, abnormal bone growth, intestinal polyposis. The results showed us that BMP signaling, mediated by BMP1a, is directly involved in inhibiting stem cell proliferation in the skin and intestine niches. They also indirectly regulate HSCs through control of their niche. (93) Noggin, the BMP4 antagonist, is transiently expressed in ISC and hair follicle areas. The coordination between Wnt and Noggin nullifies the signaling that promotes the inhibition of BMP that is necessary for the activation of stem cells. The production of intestinal polyposis supports this model due to the overexpression of Noggin. (93)

In general, the interaction between the Wnt signaling pathway and the BMP anti-growth signal is responsible for regulating homeostatic balance both for self-renewal and regeneration of stem cells. If any factor disrupts this balance, the cells may lose control over proliferation and lead to tumorigenesis. (93,98)

Chapter VI

11. Applications.

Inhibitors signalling pathways.

As has already been seen in all this work, the signaling pathways play a vital role in the proper functioning of both properties, self-renewal and proliferation, as well as microenvironment signals. Thus, these factors could inhibit such properties in cancer stem cells and thus eliminate or stop cancer progression. The most explored signaling pathways in self-renewal properties are Hedgehog, Wnt / β-catenin, and Notch. These signals have already shown promising preclinical results. Moreover, because of this, they are already in phase 1 and 2 clinical trials. (99)

Inhibition of the Hedgehog signaling pathway has a promising approach as a therapeutic target for cancer stem cells. (100) Currently, there are four models explored for the inhibition of this signaling pathway. First, SMO inhibition; second, ligand-receptor alteration; third, ligand processing inhibition; fourth, GLI inhibition. (100) Cyclopamine is a natural inhibitor of SMO; however, it has low bioavailability. Therefore, the most studied and clinically evaluated inhibitors are vismodegib, saridegib (IPI-926), BMS-833923, sonidegib / erismodegib (LDE225), LY2940680, LEQ 506, and TAK-441. In these trials, the inhibition of SMO in several tumors had favorable clinical responses. (100)

Inhibition of SMO in basal cell carcinoma (BCC) had a very favorable clinical response. In metastatic BCC, there was antitumor activity. Several studies have gone through different phases, or they are in recruitment, but preclinical responses have also been favorable. For example, in breast cancer, SMO inhibition has had favorable preclinical outcomes such as decreased proliferation, decreased tumor growth, and metastases. In chronic myelogenous leukemia, it has also had favorable preclinical results such as decreased tumorigenic potential, cells sensitized to chemotherapy. As can be seen, SMO inhibitors have been very effective in BCC housing SMO mutations. (25,100)

These preclinical studies show that Hedgehog-led treatments eliminate or decrease many vital factors in cancer stem cells. However, several markers such as Saridegib or Erismodegib, or vismodegib have shown efficacy only in certain types of cancer such as BCC or medulloblastoma (MB). On the other hand, they have had a limited response to other types of cancer, but this is still under study. (25)

Another important signaling pathway is Notch's; this signaling has excellent recognition for its potential in regulating the fate of cancer stem cells in different types of cancers (such as leukemia). It is also known for its role in shaping embryonic development. (99) In recent years, different signaling pathway inhibitors have been evaluated, such as monoclonal antibodies and γ-secretase. In 2014, Tarextumab, a monoclonal antibody from Oncomed. This monoclonal antibody has been under investigation in a study for pancreatic cancer combined with conventional chemotherapy drugs. Chemotherapy treatments are of little efficacy in this disease. The combination of tarextumab and drugs gave excellent results. 83% of the 29 treated patients had stabilization or shrinkage in the tumor over up to 12 months, which has meant a very significant breakthrough. Currently, this tarextumab treatment has already moved to phase 2 and remains promising. (25,99)

Chapter VII

12.Conclusions and recommendations.

Currently, cancer is part of the diseases with the highest mortality globally, including in our country. Stem cells are a very controversial topic that has a strong relationship with cancer. On the one hand, these cells are under investigation for cancer therapies. However, some alterations in these stem cells can lead to malignant transformation (CSCs) that may lead to cancer creation or progression. Then this is why this is important to eradicate this malignant transformation or to seek the means of this transformation not happening.

Markers used to identify cancer stem cells are also present in most normal stem cells, giving us a precedent that these two kinds of stem cells are related. The most commonly found surface markers in carcinogenic stem cells have been CD24, CD26, CD44, CD133, Ep/CAM, CD34. These markers have helped us greatly identify SC and CSC, but they are also handy for enriching or isolating CSCs. Furthermore, to sharing the expression of cell surface markers, these two kinds of stem cells share unique characteristics, such as self-renewal, differentiation, and proliferation. Essential features in both cases. In normal stem cells, these characteristics allow the regeneration and repair of different tissues or organs. In cancer stem cells, these characteristics promote tumor progression and metastasis. They are very opposite effects depending on the cell type. If malignant transformation is avoided, these cells could continue to function to benefit the human body.

Oncogenes related to self-renewal and microenvironment or niche have been two critical factors in malignant stem cell transformation. Several signaling pathways such as Hedgehog, Wnt/ β-catenin, and Notch have been involved in these two factors. Stimulation or inhibition of either pathway has had both good and bad reactions in stem cell development. And the correct functioning of the pathways has kept the cells in control. On the other hand, in the microenvironment, it could be seen that in order for the cell to perform its mechanisms correctly, they need niche signals to function, and some alteration in these signals, in the same way, can cause abnormal developments in the cell.

Inhibitions of these signaling pathways could play an essential role in inhibiting the properties of self-renewal, differentiation, and proliferation in cancer stem cells, and therefore, this could lead to the elimination or remission of cancer.

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