# Cancer stem cells, the origin of breast tumors?

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# Abstract

The origin of cancer remains an elusive feature that mainly is based on the incorporation of genetic changes during the replication cycle. There is increasing evidence that cancer initiation results from an increasing accumulation of genetic mutations in long living stem cells or their immediate progenitor cells. There are many different stem cells known and they can be divided into three main group's namely embryonic, germinal and somatic stem cells. In this paper we focus on the somatic stem cells that have the ability to self-renewal and to generate differentiated progeny for a lifetime in an organism in for example the bone marrow the breast and the brain tissue. Current evidence indicates that most cancers arise from a single cell that has undergone malignant transformation driven by frequent genetic mutations. These tumors contain rare cells with indefinite proliferative potential that drive the growth and the formation of tumors the so called cancer stem cells. Previous studies have confirmed the existence of cancer stem cells in cancers as leukemia, suggested is that breast cancer also contains cancer stem cells. These cells are characterized by their ability to proliferate and ignore the normal growth regulating mechanisms and their ability to invade healthy tissues. These cancer stem cells show many features that are also to be seen in normal stem cells. It is likely that due to the low turnover cycle and the self-renewing properties of stem cells, they have a greater chance to accumulate carcinogenic mutations compared to normal cells. This raises the question whether a cancer stem cell acquires these features from the normal stem cell or, what's also very likely, that healthy stem cells are the target for initiating cancer? There is increasing evidence that cancer initiation results from accumulative oncogenic mutations in long living cancer stem cells or their immediate progenitors, followed by the surrounding microenvironment. The microenvironment where these stem cells are located is composed of extracellular matrix, growth factors and various other support cells. The specific fate of these stem cells is also controlled by the microenvironment of the surrounding cells also known as the stem cell niche. Cancers of the hematopoietic (leukemia) system provide the best evidence that normal stem cells are the targets of transforming mutations and that cancer cell proliferation is driven by cancer stem cells. In other tissues like breast, brain or gut tissue there is evidence for stem cells but the relation of tumor development and the stem cells remains an ongoing debate. In this review we focus on the breast tissue and tumors and we find that the knowledge of the breast epithelial stem cell and the breast cancer stem cell is increasing. Several groups have found tumor populations that show the same proliferative potential as the stem cells do. The goal is to find the specific characterizations for the breast cancer stem cell and compare these differences to the normal breast epithelial stem cells. The understanding of the relationship between normal and cancer stem cells should lead to the development of new insights and strategies directed toward (breast) cancer therapeutics.

# Introduction

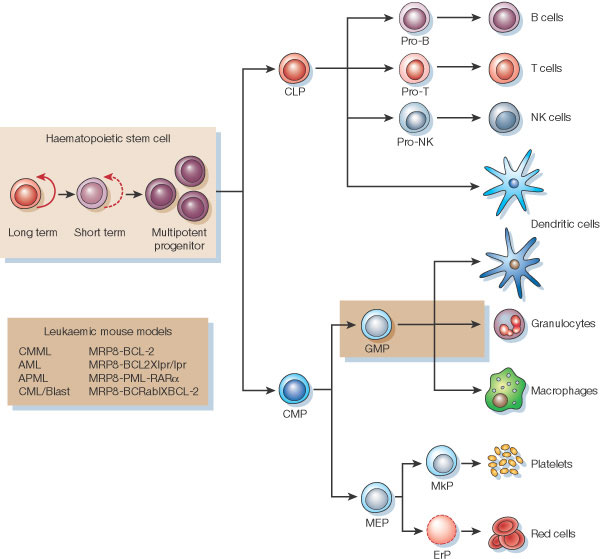
The origin of cancer is after years of research still not entirely clear, that stem cells and cancer stem cells act as kind of initiators of all sorts of cancer seems to be overall accepted. However, the way that they influence the tumor growth remains elusive. Stem cells and cancer stem cells are popular fields of research the last decades. This review presents a brief view on the recent progress in the area of cancer stem cells, with emphasis on the breast cancer cells. The fundamental problem within the cancer research is the identification of the cell type capable of initiating and sustaining growth of the tumor. The most work that has been done the last decades results in the understanding of the stem cells and the cancer stem cells in the haematological tumors. Recent studies have now shown the potential cancer stem cells in solid breast tumors, raising the possibility that such cells are at the origin of all tumors. Some studies revealed that there are small cell populations within a solid tumor, which appear to possess the same features that the normal stem cells show such as self-renewal and the ability to proliferate in phenotypic diverse (cancer) cells. Others have found different cancer cells in breast tumors and tried to characterize these cells. It seems that gradually the mystery of breast epithelial stem cells and particular cancer stem cells is being revealed. These new findings of cancerous stem cells might lead to improved diagnostics and therapies strategies. The therapeutic potential of stem cells and the growing consensus that tumors contain stem cells highlights the importance of understanding the stem cell fate and their connections to cancer.

# Cancer

Cancer has for decades been considered as a process that inflicts progressive genetic alterations in normal human cells. These genetic alterations cause normal cells to proliferate ignoring the growth regulating mechanisms and invade normal tissues. This loss of regulation has been thought to be the key to carcinogenesis. This is a multistep process that involves malfunction in proto-oncogenes, tumor suppressor genes, and other key cellular genes implicated in cell proliferation, differentiation, survival, and genome integrity (Hahn and Weinberg, 2002). These events are considered to be followed by a clonal selection of variant cells that show increasingly aggressive invading behavior. The influence of stem cells in these processes is a very plausible explanation for the existence of cancers.

## Stem cells

The evidence of the existence of cells that are able to self-renew and to generate mature cells that are needed for building a tissue is an important fact. These so called stem cells are thought to be found in every known tissue but are hard to find and hard to collect and proof their existence *in vivo.* The identification and isolation is of these rare somatic tissue specific stem cells is especially hard. There are three groups of stem cells known; the embryonic, germinal and somatic stem cells. Embryonic stem cells are derived from the inner cell mass of the blastocyst and are the ancestors of all cells in the body. Somatic stem cells are responsible for normal tissue renewal. As noted the somatic stem cells is best established in tissues with rapid cell turnover, such as skin, colon and blood. The stem cells share two major properties that are the ability to self-renewal, as well as the differentiation into mature cells in the organ that the stem cells are located. The discovery of the hematopoietic stem cell has led to a better understanding of stem cells in general and made further research possible. Hematopoietic stem cells (HSCs) demonstrate the dual ability to self-renewal and to generate daughter cells that can differentiate into several lineages that form all of the cell types that are found in the hematopoietic system. The cells that form the intermediates between HSCs and the differentiated lymphoid and myeloid cells are usually referred to as progenitor cells, **Figure l** shows the different stages of a stem cell that results in a terminally differentiated red blood cell for example. (Jean C.Y. Wang et al) One of the most important features of the stem cell is his ability to self-renewal, because it is required for maintaining all sorts of tissues. Understanding this ability in normal stem cells is fundamental also for the understanding of cancer cell proliferation, because cancer is considered to be a disease of unregulated self-renewal. Evidence shows that many pathways that are classically associated with cancer may also regulate normal stem cell development. For example, the oncogene Bcl-2, which after over-expression results in the prevention of apoptosis. This suggests that this gene regulates the apoptosis in the stem cell and when deregulated can create a cancer cell. From molecular mechanisms within the hematopoietic system underlying the development, differentiation, self-renewal, and plasticity of HSCs is still little known. Some individual studies revealed that many genetic factors were involved, such as wnt signal pathway (Reya et al. 2003), the stem cell leukaemia SCL/ tal-1 gene (Mikkola et al.), transcription factors Hoxb3 and Hoxb4 (Bjornsson et al), proto-oncogene bmi-1 (Park et al. 2003), Wilms' tumor antigen-1 (WT1) (Alberta et a), myeloid leukaemia-associated oncogene Hoxa9 (Thorsteins dortir et al), rae28 gene (Ohta et al.) The similarity between these pathways which appear to be used in cancer and normal stem cell development is an indication that these cell types are very alike but the evidence for this similarity is still scarce.

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***Figure l Development of hematopoietic stem cells****. Hematopoietic Stem cells (HSC)s can be subdivided into long-term self-renewing HSCs, short-term self-renewing HSCs and multipotent progenitors (red arrows indicate self-renewal). They give rise to common lymphoid progenitors (CLPs; the precursors of all lymphoid cells) and common myeloid progenitors (CMPs; the precursors of all myeloid cells).Both CMPs/GMPs (granulocyte macrophage precursors) and CLPs can give rise to all known mouse dendritic cells. The isolation of precursors in the hematopoietic system has allowed the generation of a series of mouse models for myeloid leukaemia (see box, lowerleft). (Reya,Tetal; Cancer,,stem cells, cancer stem cells )*

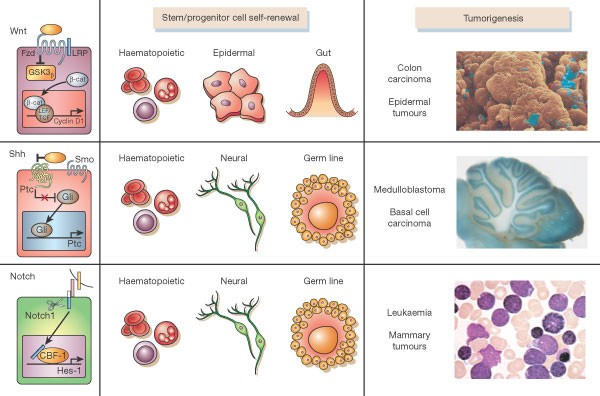
Currently there are also an number of studies that indicate the development of the tissue specific stem cells are partly regulated by the microenvironment also known as the stem cell niche (Mueller, M. M et al and Nelson, W. G et al). The concept of stem cell niche was first proposed in the human hematopoietic system in the 1970's (Schofield R et al). A similar concept has also been made for stem cells of the epidermis, intestinal epithelium nervous system and gonads (Fuchs E et al). During post-natal life, the bone marrow (BM) supports both self-renewal and differentiation of hematopoietic stem cells (HPCs) in specialized micro environmental niches. Why BM functions as a hematopoietic organ is unclear, BM provides an environment in which cells can interact, not only with each other but with osteoblasts, extracellular matrix, growth factors and various other support cells. The balance between self-renewal and commitment of stem cells is controlled by a combination of cell-intrinsic and external regulatory mechanisms. Currently, the hematopoietic niche is divided in three sections, an osteoblastic zone, a vascular zone and the cells of the neighboring HSCs (Toshio Suda et al). Cells that are known to influence the niche are the mesenchymal stem cells (MSC). These MSCs defined in literature as, the heterogeneous population of cells isolated as the adherent population when total bone marrow is placed in culture. (Bensidhoum M, et al and Bai X et al). The tumor cells also show the heterogeneous structure existing of fibroblasts, endothelial cells and inflammatory cells. These cells are called the tumor stroma and are increasingly thought to be a critical contributor to malignant growth and survival of the tumor mass, however stroma cells themselves are usually not malignant. This influence of the microenvironment in the process of carcinogenesis and the deregulation of stem cells remains elusive. Although specific conditions such as tissue injury or infection might provide signals that counteract these regulatory functions (Grandics Peter et al).

## The cancer stem cell

Stem cells in different tissues vary in respect to their ability to self-renewal and to differentiate into particular cell types. Most cancers also contain a heterogeneous population of cells with different proliferatory stages as well as differences in their ability to reconstitute the tumor after transplantation. This indicates that there are certain cells that carry the ability to self-renewal and thus to give rise to malignant cell types and therefore are able to form and maintain a new tumor after transplantation. These populations are a minority of cells within the tumor; these rare cells are called cancer stem cells because of the similarity with the normal stem cells.

These cancer stem cells have been first identified in hematological malignancies where only a few cell show the ability to form new tumors. In models of brain tumor, breast cancer and acute myelogenous leukaemia, cancer stem cells have been isolated and placed into experimental animals to form new tumors. These experiments provided strong evidence that these cells are the root cause of the tumor. Such individual cells, which are capable of self-renewal, proliferation, and differentiation to create the complex heterogeneous tumor, clearly resemble the characteristics of stem cells (Wulf et al; Al-Hajj et al; Hemmati et al; Singh et al). Because it is easier to isolate, the first evidence of the existence of cancer stem cells come from the study of the hematopoietic system. Hematologic malignancies, in particular chronic myelogenous leukaemia (CML) and acute myelogenous leukaemia (AML) have served as important model diseases in the establishment of the current knowledge of the cancer stem cell (Jean C.Y et al). This was shown using immune deficient mice models. After transplantation of the different cell types, only in a few cases the cells where able to initiate tumors. These stem cells are also identified in breast and central nervous system tumors. In solid tumors there are several models that have been proposed, two of which are the most likely; one model proposes that different cells can proliferate and form new tumors (Nowell, P et al) this model implies that proliferating daughter cells do not necessarily show the same phenotype as the parental cells. Another model shows that only the cancer stem cell has the ability to proliferate extensively and is involved in formation of new tumors. (Reya, T et al 2001) Most of the research uses hematological malignancies as model for the cancer stem cells resulting in a fairly well known system of stem cells and their deregulation in leukaemia and the bone marrow. As mentioned before the similarities are striking, intriguing is the fact that similar growth regulators and control mechanisms are involved in both cancer and stem cell maintenance. For example proteins from the polycomb group, the epigenetic chromatin modifiers, are involved in both cancer development and the maintenance of embryonic and adult stem cells (Valk-Lingbeek ME et al). In addition, pathways used by bone marrow stem cells for trafficking appear to be exploited by tumor cells for metastasis (Liang Z et al). Chemokines and cytokines have been found that are responsible for the homing of stem cells back to the BM cavity. The same chemokines and cytokines influence the migration of cancer cells. (Lyden D et al, Janowska-Wieczorek et al). Other signaling pathways associated with oncogenesis, such as the Notch, Sonic hedgehog (Shh) and Wnt signaling pathways, may also regulate stem cell self-renewal. **(Fig. 2)** (Taipale, J. & Beachy, P. A.). Groups have suggested that that Notch activation promotes HSC self-renewal, or at least the maintenance of multipotentiality (Varnum-Finney, B. et al, Karanu, F. N. et al). Shh signaling (**Fig. 2**) has also been implicated in the regulation of self-renewal by the finding that populations highly enriched for human HSCs. One particularly interesting pathway that has also been shown to regulate both self-renewal and oncogenesis in different organs is the Wnt signaling pathway (**Fig. 2**). Wnt proteins are intercellular signaling molecules that regulate development in several organisms (Nusse, R. & Varmus, H. E., Cadigan, K. M. & Nusse, R.) and contribute to cancer when deregulated. **Figure 2** shows a detailed visualization of the normal and deregulated influence of the previous named signaling pathways.

These are a few examples of the resemblance of the stem cell and the cancer stem cell but it is still not clear if these cancer stem cells are derived from true tissue stem cells, bone marrow stem cells or mature stem cells that haven undergone de-differentiation or a trans-differentiation process. (Reya et al 2001, Passegue, E .et al). It is also not entirely clear if the cancer stem cell represents one or multiple phenotypes, there even is a possibility that progenitor cells can acquire stem cell like properties, which they have lost after differentiation and after accumulation of genetic mutations, can initiate and maintain a tumor. Tumorgenesis might begin either in a primitive multipotent stem cell or in a more mature downstream progenitor. The cell of origin in different tumor types might differ owing to the unique biology of the tissues in which they arise.



**Figure 2** ***Signaling pathways that regulate self-renewal mechanisms during normal stem cell development and during transformation****. Wnt (Zhu, A. J. & Watt, F., Korinek, V. et al.), 57z/z(l3hardwaj, G. et al, Wechsler-Reya, R. J, Zhang, Y. & Kalderon, D) and Notch pathways (Henrique, D. et al, Austin, J. & Kimble) have been shown to contribute to the self-renewal of stem cells and/or progenitors in a variety of organs, including the hematopoietic and nervous systems. When dysregulated, these pathways can contribute to oncogenesis. Mutations of these pathways have been associated with a number of human tumors, including colon carcinoma and epidermal tumors (Wnt), medulloblastoma and basal cell carcinoma (Shh), and T-cell leukaemia (Notch). (Images courtesy of Eye of Science/SPL and R. Wechsler-Reya/M. Scott/Annual Reviews.)*

# Solid tumors and cancer stem cells

## Tumors in solid tissues

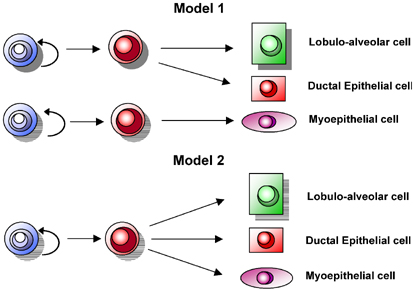
Stem cell research, as mentioned earlier, has been focused on the hematopoietic system. This has led to a better understanding and definition of details of stem cell hierarchy in the BM and blood. The progression from the most primitive HPCs to the most differentiated cells is understood in great detail and much is known about the regulation of this process (see **fig. 1**) .The knowledge of stem cell behavior in solid tumors is limited. For example, the skin and the relation to stem cells is being elucidated and the control of the progression between the stem cells and their daughter cells is just now better understood (Frye, M. et al, Owens, D. M. et al). In other tissues like the muscle tissue the stem cells have just been identified and the relation with stem cells and daughter cells is not entirely clear (Morgan, J. E et al, Seale, P. et al). In all tissues analysis of stem cell function is complicated by the presence of progenitor cells that have no self-renewal capacity but that undergo population expansion to increase the number of fully differentiated cells. Recently, substantial progress has been made in the identification and characterization of stem and progenitor cells in the mouse and human mammary gland.

## Development of the human breast

The human mammary gland changes its morphology unlike other tissues during distinct developmental stages. (Rudland, P.S. et al.) The breast tissue in humans consists of a network of ducts that form before birth by branching an invading the mammary fat pad. (Howard, B. and Gusterson, B.) A basal layer of contractile, myoepithelial cells and a luminal layer of specialized epithelial cells form the ducts. During puberty, ductal outgrowth rapidly increases under hormone stimulation, resulting in side branching. The final differentiation stage is achieved in the mammary gland during pregnancy and lactation, when numerous lobulo-acinar structures containing milk-secreting alveolar cells are formed through extensive proliferation. After suckling the lactation ends, this is accompanied by massive apoptosis and tissues remodeling and the gland reverts to a structure resembling that before pregnancy (Strange, R. et al.). To achieve this rearrangement of the tissue during pregnancy hormones, in particular estrogen, play an important role but a compartment of highly proliferative cells is needed to sustain the numerous pregnancies. This description of the highly proliferative cells matches closely to the description that is being used to characterize the stem cells or the progenitor cells.

## Mammary epithelial stem cells

In the mammary gland the presence of adult breast epithelial stem cells is still an ongoing debate. The first indication of the identification of the mammary stem cells occurred early namely around 1959. When DeOme and colleagues observed that epithelium isolated from several different regions of mammary gland was able to generate fully functional mammary outgrowths containing ductal, lobuloalveolar and myoepithelial cells (DeOme,K.B et al). In order to identify and characterize these cells within the mammary gland, several investigators have employed a variety of methods, including electron microscopy (Smith,G.H. et al),serial transplantation using limited dilutions, Southern blot analysis of unique viral integration sites (Kordon,E.C. et al) and, most recently, flowcytometry (Welm,B.E. et al). Some have suggested that the existence of breast epithelial stem cells as a fixed population is unclear and that the population has stem cell like abilities, as an effect of the interactions with the microenvironment, the niche. The evidence indicates the existence of a small sub-group in the population consisting of stem cells and progenitor cells. (Smalley, M. et al, Dontu, G. et al. (2003)) It is possible that there is no permanent specialized stem-cell type within the mammary epithelium. In this scenario, behavior such as tissue renewal and the ability to generate alveoli in response to pregnancy could be a property of all cells, removing the requirement for specialist stem or progenitor cells, and whether or not any cell responds could be random. In this case, cells might undergo a transient stem-cell-like phase for instance, under the influence of hormones during pregnancy to generate new tissue structures (alveoli or ducts). In short this is shown in the following two models (**Fig 3**). Where model one stands for a population of stem cell like cells in the breast, which can differentiate into the three different tissues (**Fig 3**). The second model the self-renewing stem cell is the precursor to all three tissues.



***Figure 3 Models of breast development hierarchy.*** *Identification and purification of the different mammary ductal epithelium stem and progenitor populations will result in a better understanding of breast cancer development. Model l depicts two different stem cells, both possessing the ability to self-renew, that can give rise to cells of the epithelial and myoepithelial lineages, respectively. In the second model, a single self-renewing stem cell gives rise to all three populations of Cells.*

There are groups who report on this second model, the so called side population cells, (Welm et al, Alvi et al) they suggest that there is a lack of evidence for the first model thus the undifferentiated cells found en the mammary gland are in fact stem cells. (Daniel CW et al, Chepko G et al). Analysis of mouse tumor virus Retroviral integration sites has shown that a complete mammary gland can develop from the progeny of a single cell (Kordon,E.C). Recently, Dontu et al. have developed a new in vitro culture system allowing the propagation of putative stem cells from normal breast tissue. In this situation, cells grow in perfect spheroids, named mammospheres, and show the two classic features of stem cells: the ability to both self-renew and to differentiate. Micro-array analysis of these cells showed expression of many genes that are similar to those expressed in hematopoietic cells, neuronal cells, and embryonic stem cells. Importantly, when overexpressed in the mammary gland, many of these genes result in tumorigenesis (Dontu, G. et al 2003a). The isolation and characterization of breast epithelial stem cells should help elucidate the molecular pathways that govern normal mammary development and carcinogenesis. According to (Alvi et al 2003) the identity of this cell is unclear, this indicates that just the last piece of the puzzle on the existence of stem cells has still to be unraveled. The general opinion between the models is divided but evidence suggests that the existence of mammary stem cells is very likely. (Frye, M. et al. Owens, D. M et al). Most theories assume that the mammary epithelial stem cells do exist and suggest that these epithelial stem cells are potential targets for breast cancer.

## Mammary epithelial stem cell niche

About the stem cell niche in the mammary gland very little is known, some studies in rats show that these stem cells are found in anatomically specialized places created by the cytoplasmic extensions and modifications of neighboring differentiated cells. Such specializations may help to regulate stem cell activity by modulating molecular traffic to stem cells and contact with signaling molecules in the basement membrane (Chepko G et al). This suggests a plasticity that may be relevant to the response of niches to tissue demands, such as wound healing, the periodic growth and regression of mammary epithelium, the process of mammary tumorigenesis and is a potential therapeutic strategy for breast cancer. Dickson, R et al used genetically engineered mice as models of ductal or lobular breast cancer that is caused by overproduction of certain proteins. These proteins (c-Myc and TGF-alpha) exist naturally in the body, but when produced in excess in mammary tissue cells, breast cancer can develop. The human stem cell niche in the mammary gland and its influence on carcinogenesis is not clear and needs more research.

## Mammary cancer stem cells

Recent studies in solid tumors indicate as mentioned earlier that the concept of stem cells in cancer might have broader implications then only the field of hematopoiesis. Most research that has been done indicates that there is one overall agreement on how breast cancer originates; there is one distinguished and rare group of cells within a tumor that can form new tumors after transplantation. This group contains cells with high proliferative potential and the ability to differentiate in a variety of cells. These differentiated cells can be found in the rest of the tumor and are unable to regenerate a new tumor after transplantation. But the characterization of these cancer stem cells in the breast remains an interesting issue that is being investigated all over the world. There are varieties of features that can contribute to the understanding of the forming of breast cancer tumors. These features can be found on various levels of development of the tumor here we view a display of features that are beginning to be elucidated.

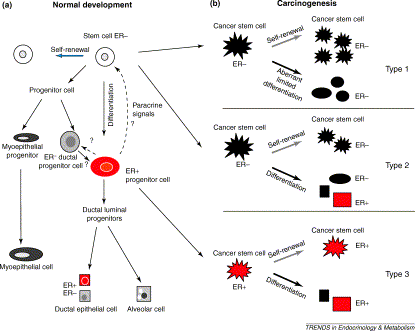
In breast tumors, a minor, phenotypically distinct tumor cell population has been isolated that is able to form mammary tumors in NOD/SCID mice, whereas cells with alternative phenotypes in the same tumor are non-tumorigenic. (Al Hajj, M. et al.(2003)) The tumorigenic cells show features that are seen in normal stem cells namely the ability to self-renewal and are also able to regenerate a heterogeneous tumor, as well as producing non-tumorigenic cells. Thus like in hematopoietic cancers, breast cancer appears to be driven by a rare population of tumor-initiating cells. Accumulation of mutations, which is the origin of breast cancer, appear to be located in cells that persist throughout a woman's lifetime. Since there is an exponential increase in breast cancer incidence with age (Woodward WA et al) it is likely that the mammary epithelial stem cells or their progenitors are the target for these mutations rather than the short lived terminally differentiated lobulo-alveolar, ductal-epithelial or the myoepithelial cells (**fig. 3**).

Al-Hajj et al (Al-Hajj M et al) reported the most concrete evidence for the existence of breast cancer stem cells. They identified a cancer stem cell population based on the presence of CD44 and absence CD24, two specific surface markers. To test the tumorigenicity of this cell population, a xenograft nude mouse model for human breast cancer was used which allowed the serial passage of tumor cells isolated directly from the patient. In eight out of nine patients, a potential tumorigenic cell population was identified and isolated by flowcytometry (FACS). It was also demonstrated that CD44+/CD24- cells lacked markers for differentiated epithelial cells and had a 10- to 50-fold increased ability to form tumors in the xenograft model compared with the original tumor mass that was found. As few as one hundred CD44+/ CD24- cells were able to form tumors in mice, whereas cells with alternate surface phenotypes failed to form tumors, even after the transplantation of millions of cells. The tumors formed by CD44+/CD24- cells possessed the phenotypic heterogeneity found in the original tumor from the patient, including born tumorigenic and non-tumorigenic cells (Al-Hajj M, Clarke MF (2004)).

More evidence for the hypothesis that normal stem cells are the target for tumorgenesis in the adult mammary gland, and form the tumor stem cell population comes from Boulanger and Smith and co-workers. (Boulanger, C. A.et al) The reduced fat-pad repopulation ability of mammary epithelial cells that are derived from transgenic mice that carry a TGF-transgene under the control of the whey acidic protein (WAP) promoter was interpreted as being due to premature stem-cell senescence. These animals were more resistant to tumorgenesis that is induced by the mouse mammary tumor virus (MMTV), compared with wild-type animals. In the tumors that did develop, the WAP-TGF beta transgene was active, indicating that tumorgenesis had not been affected by presence of the TGF-beta protein, but rather by stem-cell senescence. (Boulanger, C. A.et al)

Emerging evidence also links the alteration of Wnt (**Fig. 2**) signaling pathway to the transformation of mammary progenitor cells into cancer cells (Li et al. (2003);Liu et al. (2004)). In addition, an important susceptibility gene for breast cancer, which is called BRCA1 and confers substantial lifetime risks of breast cancer, was recently found to be acting as a stem cell regulator (Foulkes et al 2004).

The risk of developing breast cancer is increased in woman how have an early pregnancy. There is evidence that this is induced by the hormonal influence of estrogen during the development of the adult mammary gland. Substantial progress has also been made in the identification of the involvement of the estrogen receptor in breast cancer subtypes. (Gabriela Dontu, Dorraya El-Ashry et al) The importance of estrogen mediated responses for normal development and growth of the mammary gland is well documented by studies in humans and animals. (Bocchinfuso, W. and Korach, K et al, Anderson, E et al.) The influence of estrogen and its receptor in mammary carcinogenesis is well recognized. There is hypothesized that deregulation of normal self-renewing cells could lead to increased symmetrical cell divisions which would explain the proliferation of steroid-receptor positive cells in cancerous breast lesions. (Gabriela Dontu et al 2004) This has lead to the development of a model (Fig 4) for the existence of Estrogen Receptor (ER) in slow dividing stem cell populations in the mammary gland. These data suggests an alternative explanation for the segregation of steroid receptors and proliferation markers. Rather than being terminally differentiated cells, steroid receptor positive cells might represent undifferentiated stem or progenitor cells that in-vivo are mostly quiescent or slowly dividing. These cells give rise to more rapidly proliferating cells, which lose their steroid receptors in addition to terminally differentiated, non-cycling ER+ cells. (Gabriela Dontu et al 2004)



***Figure 4.******Mammary development, carcinogenesis and the estrogen receptor (ER), (a) During normal development mammary stem cells undergo self-renewal and differentiation.*** *The primitive progenitor cells, general during fetal development of the breast, give rise to myoepithelial progenitors and ER- progenitors, which form rudimentary branching structures, independent of estrogen stimulation. ER+ progenitors arise after 30 weeks of gestation, and respond to estrogen stimulation by proliferating and generating ductal epithelial cells and alveolar cells that form the adult mammary tree. Paracrine signaling initiated by the ER+ progenitor cells might also be involved in maintaining the myoepithelial progenitor pool and the stem cell compartment. (b) Carcinogenesis is the result of mutations affecting various progenitor cells. The phenotype of different subtypes of breast cancer is determined both by the cell of origin and the particular mutations driving carcinogenesis. Thus, ER- tumors arise from stem cells or early ER-progenitor cells, whereas ER+ tumors can arise from either ER-progenitors or ER+ progenitors. (Gabriela Dontu, Dorray El-Ashr et al.)*

During the rudimentary development of the mammary gland estrogen does not influence the gland cells only during pregnancy development of the gland multiple cell compartments are influenced by estrogen. This suggests as shown in **figure 4** that all cell populations progenitor cells as well as the stem cells after rudimentary development of the gland are ER+ and sensitive to estrogen. The initial rudimentary mammary tree can develop in an estrogen independent way. (Bartow, S.A et al.) The consequences on the level of carcinogenesis are shown in the second part of **figure 4**. The type one tumor shows a block in differentiation upstream of ER+ progenitors. There is a majority of cells that show the absence of the estrogen receptor and the differentiation rate is very low. This has consequences for the treatment and the category of tumors that originate, namely that the tumors are aggressive and have a poor prognosis; the treatment with ER modifiers has a minimal result. The second type of tumors show a variable expression of the ER, from very high to very low. Using anti-estrogen treatment results in an initial shrinking of the tumor but will not have a long lasting effect because the anti-estrogen does not affect the ER- cells. The third group of tumors shows almost solely expression of the estrogen receptor on the cells. This group has the best prognosis and benefits the most from hormonal therapy. The fact that makes this model highly plausible is that this model accounts for the variation of the clinical results within patients during hormonal treatment strategies in the clinical field. Future studies involving the transcriptional profiling of purified populations of normal and malignant stem and progenitor cells should provide a direct test of this model.

# Relevance of understanding cancer stem cells

Cancerous stem cells share the same properties of self-renewal and differentiation with normal stem cells, with addition of similar phenotype of adult stem cells. The existence of these cancer stem cells is still under debate but the majority of groups have accepted the fact that they exist and that they influence the initiating tumor. The origin however remains not very clear and some believe that the cancer stem cells are derived from mutation of the normal stem cells, whereas others suspect it to be from a different origin. Further investigation of adult stem cells and the cancer stem cells will shed light on the fïelds of origin and behaviour of these essential cell types. Consensus of the existence of cancer stem cells in the breast and other tissues may provide new insights in understanding some general behaviour of tumors and result in new strategies for cancer therapy. Think of the fact that cancer stem cells form only a small proportion of the total tumor size but make sure that the tumor can keep growing. This small group of slow dividing cells might explain why so many cancers regenerate after treatment with irradiation or cytotoxic drugs, even when the majority of the volume of the tumor seems to be killed. Residual cancer stem cells may survive in a quiescent state for many years after remission and result in later relapse and metastasis.

Current insights show that cancer stem cells and the normal stem cells appear to share the same self-renewal pathways. Therefore, it will be important to understand the biology of normal tissue stem cells in order to find the changes, which might occur in cancer stem cells. As shown in the hematopoietic system the leukaemia stem cells may be heterogeneous with respect to their self-renewing potential and their quiescent state. This is very likely to be true in solid tumors.

Characterization of these cancer stem cells but also of the normal stem cells in breast tumors and other solid malignancies might provide the knowledge that is needed to create specific drugs, targeting these cancer stem cells. It may be necessary to profile the tumor stem cells in the different breast cancers to find the appropriate therapeutic target. As shown this research is already been done by a lot of groups, in this review we focused on the stem cells and cancer stem cells found in breast and breast tumors. It is still not clear whether the identified cancer stem cells express the same phenotype or surface markers as the breast tissue stem cells. There are many indications about the composition of the breast cancer cells for example the two surface markers found by Al-Hajj et al. In addition, the model (Fig 4) explaining the potential importance of the existence of the Estrogen Receptor. The observation that a self-renewing cancer cell population drives tumor formation suggests that identification of agents that specifically inhibit cancer stem cells self renewal or survival while sparing normal stem cells and other essential normal tissues would result in more effective and less toxic treatments. These findings along with other characterizations might lead to the understanding of the origin of breast tumor and other solid tumors and the involvement of cancer stem cells.

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