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Breast Cancer Stem Cells – A Review

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1. Introduction

Breast cancer is a leading cause of morbidity and mortality among women. Much of the morbidity and mortality is associated with metastasis or local recurrence. There has been significant research on what can cause breast cancer progression and resistance to treatment. Carcinomas are made of transformed cells contained within stromal cells, fibroblasts, and immune cells. Difficulty in determining definite causal pathways of development of resistance and metastases is complicated by the heterogeneity of breast cancers. There are many different subtypes of breast cancer, each with certain unique phenotypes and genotypes. There has been shown 3 subsets of mammary cells: basal stem/progenitor cells, luminal progenitors, and non stem luminal cells. Although there is heterogeneity among the cell population of breast cancer tumors, there is generally a dominant cell type that allows classification of the tumor. There are at least four different breast cancer phenotypes. The first is the normal-like phenotype which resembles non cancerous breast cancer. The second is the luminal phenotype, generally ER positive and often divided into luminal A and luminal B categories. These breast cancers also express E-cadherein, KRT8, KRT18, and KRT19. The third category is HER-2 positive phenotype, which is generally ER negative. The fourth phenotype, designated as basal-like, generally overexpresses markers characteristic of normal mammary gland myoepithelium including EGF, p63, and basal cytokeratins KRT14, KRT17, and KRT5/6.

However, a poorly differentiated phenotype has been established as a hallmark for aggressive breast cancers, which has suggested a role of stem cells in breast cancers. Stem cells are defined as cells that have the ability to undergo unlimited cell cycle divisions to create new stem cells that retain their undifferentiated state and multipotent differentiating potential. There are two broad categories of stem cells, embryonic and adult, or somatic, stem cells. Embryonic stem cells are found in the inner cell mass of blastocysts and involved in the differentiation of the embryo. Somatic stem cells are found in various tissues after embryogenesis with the main function of normal tissue repair and renewal. Examples include mammary stem cells found in breast tissue and recently associated with breast carcinomas, as well as mesenchymal stem cells, found in various locations throughout the body, including the bone marrow, and involved in modulating immune response. Many believe that the somatic stem cells are not as pluripotent as embryonic stem cells and should therefore be considered more as progenitor cells rather than stem cells. Progenitor cells are similar to stem cells in their ability for self-renewal and ability to differentiate into multiple

cell lineages but with a finite number of cell cycle division and more drive toward differentiation than embryonic stem cells. Somatic stem cells and progenitor cells are often used interchangeably, as will be the case in this chapter.

An increase in interest in stem cells began in 1997 when malignant stem cells were found in acute myeloid leukemia. Subsequent research revealed stem cells in various other solid tumors such as prostate, breast, lung, colon, and brain. The discovery of stem cells in breast cancer along with the heterogeneity of breast cancers and ability to develop resistance to treatment and relapse helped change the previous theory of each cell having equal tumorigenic potential to a preference for a theory suggesting that only certain cells within the tumor population allow progression of the tumor. Where these stem cells came from, however, is still up for debate. Breast cancers have a very heterogeneous set of phenotypes which leads to problems with generalizations of clonal origins of breast cancer stem cells (BCSC). As will be discussed in this chapter, there are several theories about breast cancer stem cell origins. Research about BCSC has captured the attention of scientists because of the undifferentiated character of aggressive breast cancer and the ability of stem cells for self-renewal, which may fuel breast cancer tumor progression and metastases. However, there are significant complicating variables that must be taken into consideration in BCSC research. BCSC are complicated with unknown origin, various pathways linked with a variety of physiologic processes as well as the uncertainty of isolating breast cancer stem cells due to lack of definite cell markers and BCSC definition. An additional roadblock in furthering research on BCSC is the possible need of these cells for a particular niche in which to grow. Not only must research concentrate on the characterization and pathways of BCSC but also take into account the normal physiologic processes and environmental conditions which influence the behaviors of cells and tissues . Additionally, it remains to be determined whether different subtypes of breast cancer harbor different types of BCSC.

In evaluating breast cancer, factors to be considered include subtype of breast cancer, prognosis, what causes relapse and why breast cancer tumors can develop resistance to therapy. Although stem cells have been suggested to be the culprit for possible relapse and resistance to therapy, which is often linked to a poor prognosis, the presence of stem cells alone is not indicative of poor prognosis. Indeed, mammary stem cells are found in normal breast tissue, shown by studies to be present in the basal epithelial compartment of mammary glands. Therefore, it is paramount to determine why stem cells become malignant or from where the malignant stem cells came. Despite that the exact nature and role of BCSC still remain elusive to researchers, much has been discovered about them since 1997, with every day giving more insight into their role in breast cancer. This chapter will give an overview of the major players in BCSC research in characterization, pathways, and treatments, all suggesting possible future directions in breast cancer research.

2. Characterizing breast cancer stem cells

One of the difficulties in research about BCSC remains the elusive definition of the cells. Although there is no concrete definition of cancer stem cells, generally, scientists include the following two characteristics in determining BCSC: the ability for self-renewal to generate another malignant cell and the ability to show lineage-specific differentiation. Even given these criteria, characterization of BCSC is further hindered by phenotypes linked to self-renewability such as ALDH1. These phenotypes will be further discussed later in the chapter. Other universally accepted characteristics of BSCS include undifferentiated

phenotype and resistance to various breast cancer therapies. The resilience of these cells to less than optimal conditions has potentially been attributed to their low mitotic rates, when compared to the mitotic rates of their differentiated counterpart.

An important characteristic of stem cells is their inherent resistance to multiple drugs. This multiple drug resistance (MDR) profile is generally believed to be conferred to these cells mainly by ATP-binding cassette transporters. BCSC are thought to be associated with overexpression of these transporters and the most likely mechanism for failure of chemotherapy in these cells (Figure 1). The ATP-binding cassette transporters are able to efflux various, unrelated drugs out of cells on which they are present, thus conferring an MDR profile to these cancer cells. Multidrug resistance gene 1(MDR1) also is a major cause of breast cancer resistance to chemotherapy. Other multi drug resistance-linked genes include ABCB1, CCNE1, and MMP9. Although MDR is a significant obstacle to effective chemotherapy, MDR appears to be a normal protective function not only in malignant cancer cells, but also in their benign counterparts as studies have identified ATP-binding cassette transporters in normal tissue stem cells. Importantly, MDR activity is up regulated in response to chemotherapy. Of significant concern, these transporters have also been found in tumor cells that have not been exposed to chemotherapy, indicating a built in obstacle to antineoplastic treatment. Studies have shown that chemotherapy also

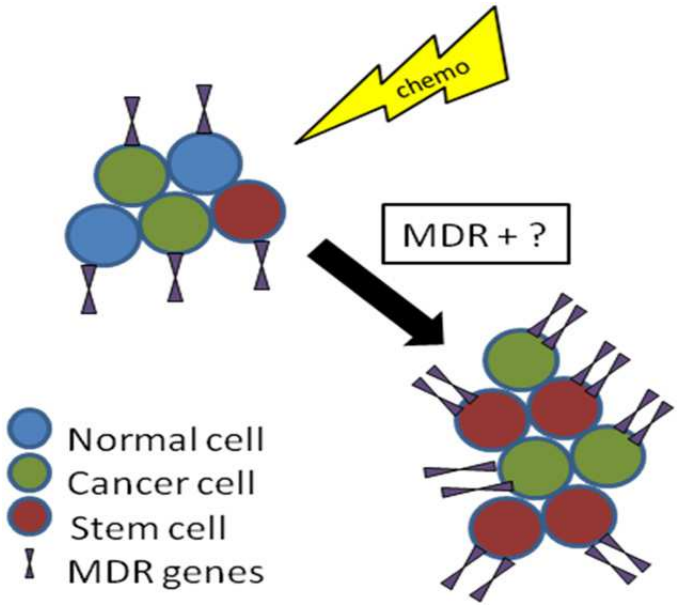

Fig. 1. MDR gene in breast cancer.

MDR is a natural defense mechanism in many, non-carcinogenic cells. However, MDR genes can be upregulated when exposed to chemotherapy, which can allow for tumor proliferation and an increasing stem cell phenotype. Nonetheless, expression of MDR genes is not the only factor in determining cancer progression or induced-stem cell phenotype after chemotherapy because sole upregulation of these genes do not result in stem cell phenotype, only treatment escape.

significantly increases tumor enrichment with stem cells, suggesting the idea that MDR genes may be involved in stem cell phenotype. However, singularly over expressing MDR genes showed that although MDR genes may allow escape from treatment, overexpression alone does not cause an increase in stem cell phenotype.

To help determine a concrete definition for BCSC, researchers have endeavored to find cells markers common, or more helpfully, exclusive, to BCSC. Cell markers that have garnered significant interest in research include CD44 and CD 24. CD44 is a transmembrane glycoprotein which is involved in cell adhesion and migration and has been shown to be upregulated in various cancers as well as their metastastes. Studies in which the blockage of CD44 led to inhibition of local growth and metastases suggest that CD44 is potentially a protective measure in breast cancer tumors. CD24 is a heavily glycosylated cell marker, which has been suggested to play a role in tumor migration due to its ability to bind P-selectin, a lectin expressed by endothelium and platelets. It has been suggested that the dynamic nature of the CD marker expression, however, prevents CD44 and CD24 from being definite markers for BCSC. Their expression can be influenced by epigenetic factors, genomic instability, and epithelial to mesenchymal transition, discussed later in this chapter. In addition, other studies have found that CD24 is not a consistent marker for breast cancer even though it has been and still continues to be used as a marker for BCSC. Therefore, the tide is potentially turning away from using these as the only markers for BCSC. However, they are still currently being used extensively by the scientific community to help identify BCSC. The usage is supported by studies which show that although the CD markers were not found to be consistently expressed in all breast cancer or with an increased stem cell-like phenotype, in mammospheres, the stem cell phenotype was present as was the ability to differentiate into luminal and basal phenotypes in human breast cancers.

Mammospheres are discrete clusters of cells that have the ability to survive and proliferate in non adherent, non differentiated culture conditions and are indicative of stem cell-like characteristics. Studies have shown that mammospheres may be more accurate in studying stem cells as more stem cell markers and characteristics were found in the spheroids rather than the adherent cultures. The spheroids were also found to be more malignant with greater altered chemo sensitivity than their adherent counterpart. Therefore, mammosphere formation may be used as a characteristic to help identify BCSC. These studies additionally showed that MAPK, Notch, Wnt genes, and aldehyde dehydrogenase (ALDH) are all over-expressed in breast cancer mammospheres. These markers and pathways have all been linked to BCSC by various studies and are accepted as potential BCSC markers.

Side populations are being investigated as a potential identification method for BCSC. Side populations were originally used to establish a population of hematopoietic cells enriched in hematopoietic stem cell. They are defined by their ability to efflux the dye Hoechst 33342 out of the cell determined to be due to ATP-binding cassette transporters. Importantly, this side population was also found in breast cancers and irradiated breast cancer tumors were discovered to contain side populations enriched in progenitor cells. However, the usage of this potential method of identification is hampered by the toxicity of the dye to non-side population cells.

ALDH1 is becoming more important for isolation of BCSC along with usage for identification of side populations of breast cancer cells with MDR proteins. ALDH1 is a member of a family of ALDH enzymes involved in the detoxification of a wide array of aldehydes. Functional enzymatic assays are utilized to detect the presence of ALDH enzymes due to the wide array of enzymes within the family. There are some contradictory

studies about the frequency of ALDH+ cells within a tumor with one study suggesting it is as low as 25% while another study reported finding ALDH+ cells in 23 out of the 33 breast cancer tumors tested. Although these studies may appear contradictory in the prevalence of ALDH+ cells in breast cancer tumors, studies have shown that ALDH- cells are far less tumorigenic than ALDH+ cells, especially the small subset that also displays stem cell markers CD44+/CD24-/low. In addition, the ALDH+ tumor exhibited preference for forming high-grade, HER2+, hormone receptor negative tumor, all indicative of poor overall prognosis. ALDH1 has also been rumored to be an independent prognostic factor in predicting metastases in inflammatory breast cancer with the associated BCSC having the ability to reconstruct the heterogeneity of the originating breast cancer at the distant site .

CD133, also known as Prominin-1, has been suggested to identify a subset of BCSC. A transmembrane glycoprotein, CD133 has been used in defining a wide array of somatic stem cells as well as being elevated in the peripheral blood of patients with metastatic disease. CD133 is considered a very important stem cell marker despite not much being know about it because of it greater restriction to cancer stem cells unlike CD44 and ALDH. Additionally, downregulation of CD133 has been shown to decrease cell growth, cell motility, ability to metastasize, and ability to form spheroids in stem cell-like conditions (Figure 2). CD133 may

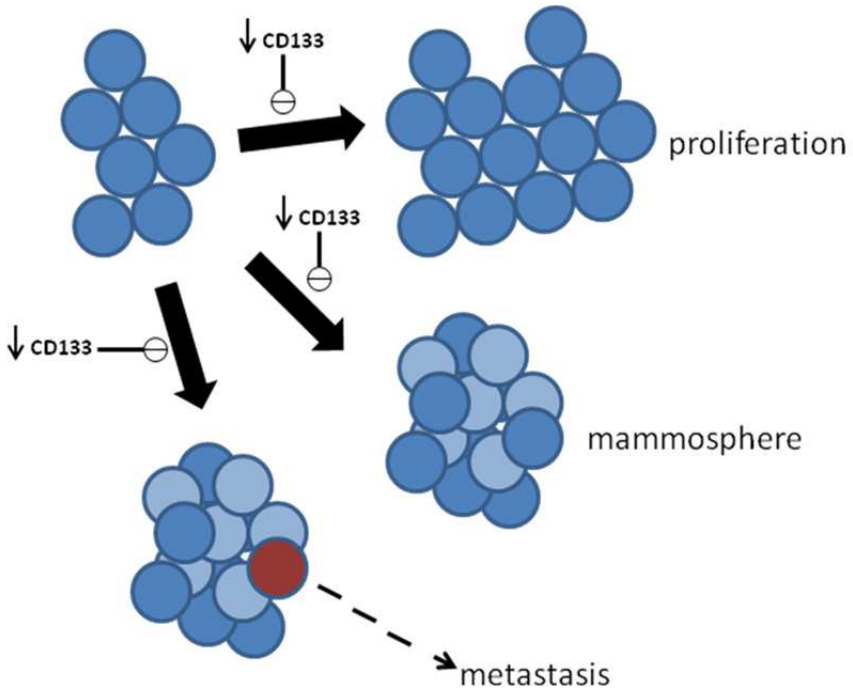

Fig. 2. Role of CD133 in breast cancer.

CD133 downregulation results in poor mammosphere formation, less metastasis, and less proliferation, suggesting a potential target for breast cancer treatment. Furthermore, CD133 is more specific to breast cancer stem cells than many other markers such as CD44, showing additional importance in the role of cancer progression and characterization.

also be a successful anti-neoplastic therapeutic target as shown in hepatocellular and gastric cancers in addition having a role as a cancer stem cell marker. CD133 is potentially a very important cancer stem cell marker in breast cancer specifically as shown by several labs. Cells from basal-like breast cancer and mammospheres from ductal carcinomas express high levels of CD133. Isolated CD133-specfic breast cancer cells from BRCA-1 lines have greater colony forming efficiency and increased proliferative potential, similar to stem cells. Finally, CD133 has been identified in a majority of inflammatory breast cancers, an aggressive form characterized by extensive lymphovascular invasion. A specific xenograft model of inflammatory breast cancer, MARY-X, has not only been shown to contain BCSC-enriched spheroids expressing CD133, but these spheroids have also been shown to contain BCSC profile of CD44+/CD24-/low and ALDH+.

3. Origin of breast cancer stem cells

The origins of BCSC to date still have not been fully elucidated. Indeed, prior to interest in stem cells, the theory of breast cancer progression was different as well. The traditional theory of breast cancer progression hypothesized that each breast cancer cell has the same tumorigenic potential and phenotypic heterogeneity, according to this stochastic model, was due to the accumulation of genetic insults in the progenitor cells. As more light was shed on the undifferentiated nature of aggressive breast cancer tumors and development of therapeutic resistance, a new hierarchical model was suggested. This newer theory believes that the progression of breast cancer is due to a subset of cells within the tumor with stem cell-like characteristics of self-renewal and multi-lineage differentiation potential, which also accounts for the heterogeneity of breast cancer (Figure 3). Further support of the hierarchical model is additionally shown by studies in which only a small portion of cells within a tumor is shown to lead to tumorigenesis. Moreover, the majority of breast cancer cells are very inefficient at tumorigenesis at a cellular level, therefore, only a subset within the tumor must be responsible for metastases by invading blood vessels and transversing the basement membrane. Within the hierarchical model, the subset of cells within breast cancer tumors leading to tumorigenesis has been suggested to be BCSC. This returns to the question about where these cells come from. There are a variety of theories on the subject. BCSC have been supposed to come from de-differentiation, progenitor cells or acquirement of genetic alterations by normal resident cells. Embryonic stem cells have been known to have undifferentiated phenotype and ability for multi lineage differentiation. BCSC are not quite the same although they have the same basic characteristics. In fact, the molecular mechanisms involved in the genesis of BCSC appear to point to a variety of pathways common to both stem cells and cancer biology, which will be discussed later. P53, a long known cell cycle regulator, has been identified as a key determinant in stem cell-like characteristics in breast cancer tumors. The mechanism by which p53 endows breast cancer tumors to develop tumor progression is by allowing reprogramming of tumor cells to become induced-pluripotent cells (Figure 4). These p53 mutations arise often late in tumor progression. There is a clear association between p53 inactivation and the presence of stem cell associated transcription factors in breast cancer. Furthermore, there is an increased incidence of decreased p53 function in malignant tumors, which implies that decreased p53 function leads to phenotypic plasticity and reprogramming of tumor cells. Additional support is seen in studies where induced p53 function led to an inhibition of induced-pluripotent cells from their differentiated counterpart. In addition, the involvement of

epithelial to mesenchymal transition (EMT), discussed below, has been purported to be involved in the creation of BCSC.

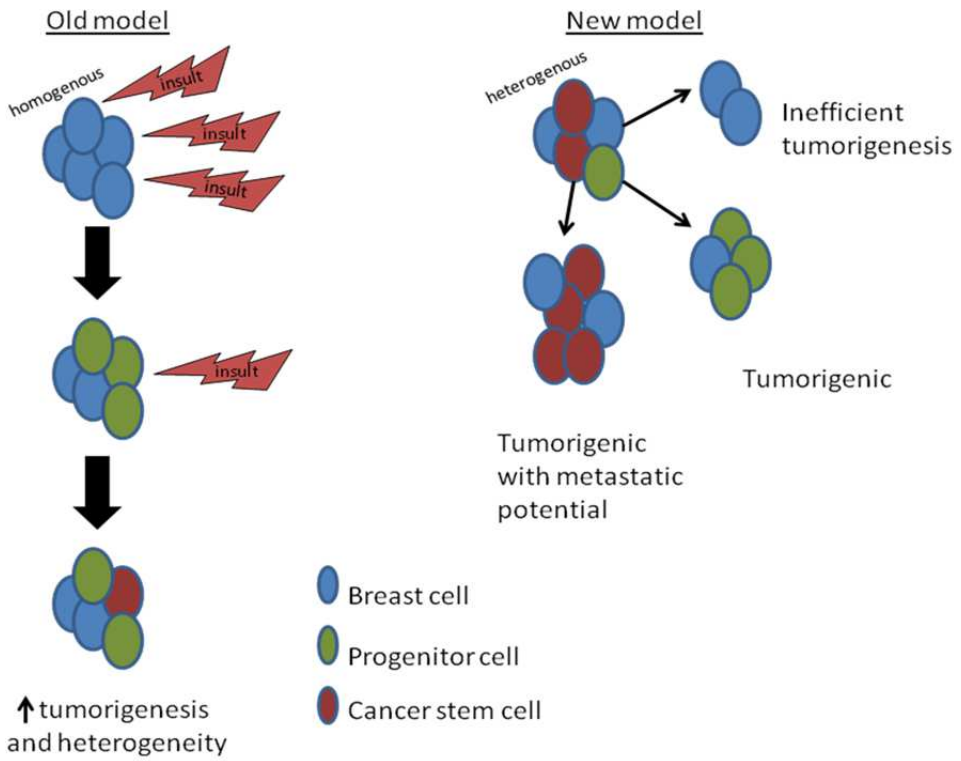

Fig. 3. Theories of breast cancer progression.
The old model of breast cancer progression assumed that all breast cells have equal potential to become tumorigenic with repetitive insults. The new model contends that the breast cells are heterogenous from the start with differing potentials for tumorigenesis, thus resulting in heterogenous breast cancers.

Epithelial to mesenchymal transition is defined as the loss of epithelial characteristics of a cell to adopt a more mesenchymal phenotype. EMT is a function of normal development, for example, involved in gastrulation of a chicken embryo. However, the mesenchymal phenotype typically allows more cell migration and invasion and is the reason EMT is being researched in relation to cancer progression and metastases. Although EMT has been researched for the past two decades, evidence showing EMT in vivo has been controversial. EMT has been identified in breast carcinomas and associated with poor prognosis at both the gene and subtype level. Features characterizing EMT include loss of cell to cell adhesion via E-cadherein in adherens junctions, occludins and claudins in tight junctions and desmoplakin in desmosomes. Aditionally, there is a down regulation of epithelial cytokeratins (KRT8, KRT18, KRT19), upregulation of mesenchymal proteins vimentin and ACTA2. Importantly, there is increased potential for migration and resistance due to an

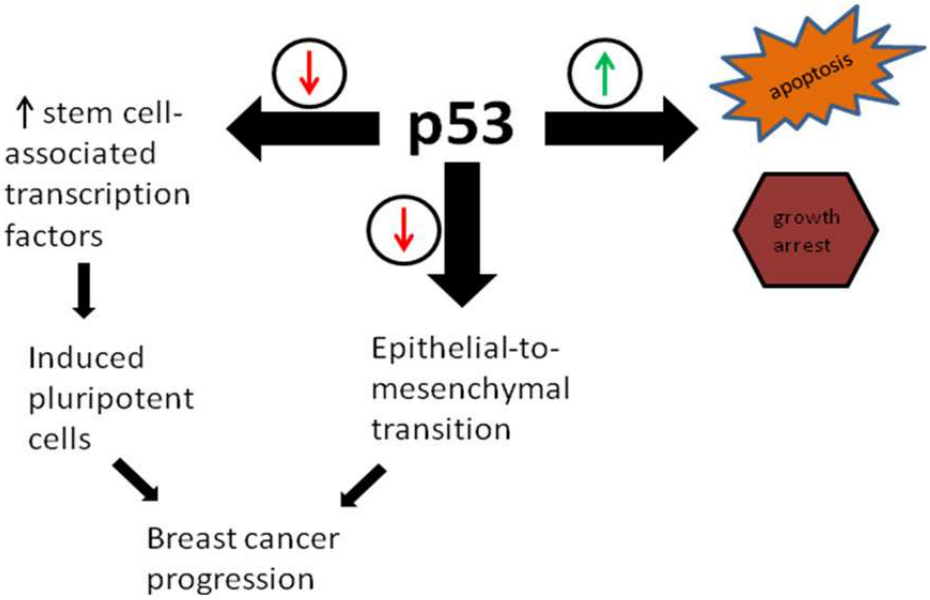

Fig. 4. p53 in breast cancer progression.
P53 is integral in regulating the normal cell cycle. Ordinarily, it eventually induces apoptosis and growth arrest through various caspases. With loss or mutation of p53, EMT and increased stem cell associated transcription factors result in breast cancer progression.

adoption of dynamic actin microfilament networks and increased resistance to apoptosis. EMT has also been shown to be induced by EGF, IGF-1, IGF-2, and TGFb, as well as though transcriptional control of E-cadherin by transcription factors such as SNA1, SNA2, ZEB1, ZEB2, TWIST, and GSC. Signal pathways such as Wnt, Hedgehog and Notch have also been implicated in EMT.

Cells that are the result of EMT and BCSC are not necessarily the same thing. EMT is considered distinct from BCSC generation but has some overlapping factors such as EMT cells demonstrate a CD44+/CD24-/low phenotype similar to BCSC. It has been suggested that EMT can help create BCSC as shown in studies where overexpression of Snail or TWIST lead to the creation of cells with CD44+/CD24-/low expression. EMT has been investigated in breast cancer and BCSC research because these cells have been associated with tumor metastases as well as aggressive breast cancer and a poor prognosis. Furthermore, EMT is implicated in the de-evolution of normal mammary cells to acquire stem cell-like properties. EMT is important in breast cancer research not only as a possible origin for BCSC but also for prognosis. EMT that may create BCSC promotes the development of refractory and resistant breast cancer because BCSC are often resistant to many cancer tx, which ultimately leads to breast cancer relapse. EMT can be induced by CD8+T cells with the resulting tumors having CD44+/CD24-/low phenotype, potent tumorigenicity, ability to re-estabilish an epithelial tumor and increased resistance to therapy further lending credence to the idea that EMT is involved in BCSC generation.

Not only do BCSC lend breast cancer the ability for tumor progression but breast cancer is also potentially affected by mesenchymal stem cells (MSC). MSC may have a role in being

protective for BCSC, thus affecting breast cancer progression. These cells have been shown to migrate to breast cancer tumor site and allow the breast cancer to become resistant to therapy. Importantly, MSC play an important role in helping BC cells evade the immune system, thus allowing tumor progression. MSC by itself has the ability to promote tumor progression by creating cancer associated fibroblasts (CAFs). These CAFs, characterized by SDF-1 are established in tumor stroma and leads to the creation of metastases, angiogenesis and other pro-tumorigenic factors; however, further details are beyond the scope of this chapter.

4. Pathways

Important pathways that have been implicated in BCSC include Hedgehog, Notch, Wnt, p53, and TGFB**.** TGFB, an immunosuppressive cytokine that is involved in wound healing, fibrosis, and cell cycle regulation has been shown to be critical in BCSC behavior. Importantly, loss of TGFB may enhance breast cancer motility (thus leading to metastases) via EMT, as discussed earlier. This increased motility may be due to the fact that TGFB is responsible for cell cycle inhibition and blocking de novo cancer formation. Loss of TGFB is associated with an increase the Sca1 marker, showing an increase in the luminal progenitor cells within the tumor. Additionally, TGFB results in a decrease in side population cells, characterized by the ability to efflux Horest dye, which is thought to be enriched with progenitor cells. Furthermore, TGFb was silenced in a cell population with the CD44+/CD24-/low phenotype, thus further supporting the fact that TGFb is a tumor suppressor.

Wnt pathway involves secreted growth factors involved in a wide range of cell processes and has been shown to be regulatory in nature of stem cell maintenance and carcinogenesis. There are two broad categories in this pathway: canonical and non-canonical pathways. The canonical pathway, including Wnt1, is b-catenin-dependent while the non-canonical pathway, including Wnt5a, is b-catenin-independent. The canonical pathway is associated with stem cell maintenance or expansion by suppressing differentiation and promoting self-renewal. Exogenous administration of Wnt to normal mammary stem cells results in an expansion of stem cells with an increase in self-renewal ability. Furthermore, Lp5, a receptor for Wnt signaling, is present in the same location as mammary stem cells in the basal-epithelial compartment of the mammary gland, as previously mentioned. A decreased in Lp5 results in the loss of stem cell activity in the mammary gland. The canonical pathway is b-catenin dependent, as previously mentioned, therefore, it follows that a gain of function mutation resulting in increased b-catenin activity results in increased mammary stem cell self renewal. Although the canonical Wnt pathway is involved in stem cell maintenance, it has also been implicated in tumorigenesis from stem cells and luminal progenitor cells as shown in studies where there is an increase in stem cells and Sca1, a marker for luminal progenitor cells, in tumors with overexpressed Wnt1. On the other hand, decreased Wnt5a in breast cancer tumors have been implicated in early relapse and poor prognosis. Wnt 1 of the canonical Wnt pathway is unregulated and Wnt5a is downregulated in breast cancer cells when compared to normal mammilary cells, indicating that the Wnt5a has tumor suppressive ability similar to TGFb (discussed above). The two opposing Wnt pathways appear to exert effects on each other to maintain normal cellular function. When the canonical pathway is suppressed by the non-canonical pathway, there is a decrease in stem cell phenotype.

Importantly, the Wnt pathway and TGFb are connected since TGFb tumor suppressive function appears to involve antagonism of the canonical Wnt pathway by Wnt5a. The two are interconnected further because TGFb regulates Wnt5a expression in mammary gland while Wnt1 of the canonical pathway mediates TGFb effects on branching during breast development. The data suggests that TGFb and Wnt5a can inhibit the canonical Wnt pathway, redirecting the mammilary tumor cells to adopt a more basal-like characteristic. The mechanism for this tumor suppression by TGFb has been reported to be due to the fact that TGFb acting through Wnt5a inhibits b-catenin, thus initiating tumor suppression by limiting stem/progenitor cell populations (Figure 5).

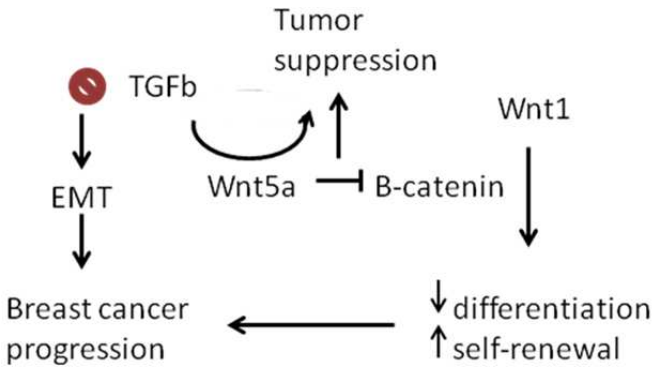

Fig. 5. TGFb and Wnt pathways interplay in breast cancer.
TGFb and Wnt5a both act as tumor suppressors. TGFb, when inhibited, induces epithelial-to-mesenchymal transition, leading to breast cancer progression. Wnt1 is normally inhibited by Wnt5a, leading to suppressed ability for self-reneal and de-differentiation. When b-catenin is not inhibited by Wnt5a, Wnt1 leads to increased self renewal and decreased differentiation, both of which lead to breast cancer progression. Therefore, Wnt1 and Wnt5a, along with TGFb, work against each other to help maintain normal cell physiology.

The hedgehog pathway has been implicated in sustaining cancer stem cells through self-renewal. The pathway was first discovered in Drosophila melanagaster and is a major regulator of cell proliferation, differentiation, and stem cell maintenance. The link to cancer was established while studying a rare familial disease, Gorlin syndrome, in 1996. Secreted Hedgehog ligands bind their transmembrane receptor Patch, which causes Smo release and dissociation of transcription factors Gil1, Gil2, and Gil 3 from Fu and SuFu. These transcription factors lead to transcription of cyclin D, cyclin E, Myc and EGF factors, enhancing carcinogenesis. In the absence of the Hedgehog ligands, the transmembrane receptor Patch associates with Smo, effectively blocking Smo function. Thus, Hedgehog inhibitors, such as cyclopamine, are being looked at as potential anti-neoplastic agents (Figure 6). Additionally, TFGb has been demonstrated to upregulate factors in the Hedgehog pathway. Further support of a role in cancer is shown by studies in which the hedgehog pathway has been implicated in progression from non-invasive phenotype to invasive phenotype in ductal carcinoma. An important fact of which to take note, however, is the fact that Hedgehog is also intimately involved in normal developmental processes as well, causing potential difficulties implementing Hedgehog inhibitors as anti-tumorigenic agents.

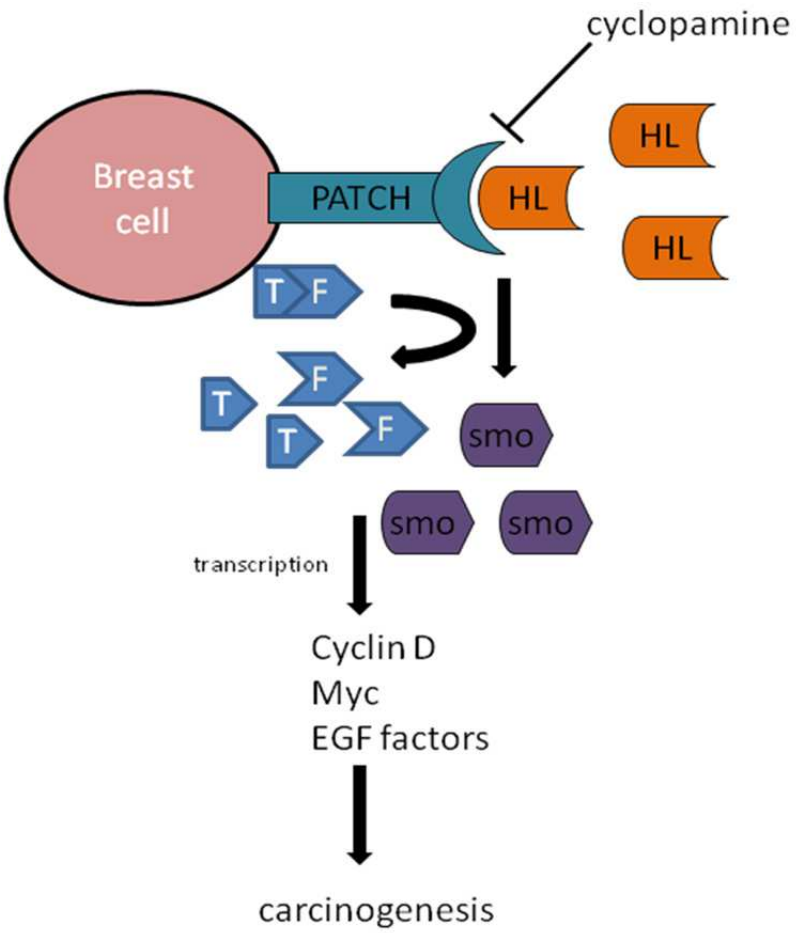

Fig. 6. Promotion of carcinogenesis through Hedgehog.
With Hedgehog ligand association with PATCH, smo is released, allowing smo to function causing dissociation of transcription factors. This causes an increase in transcriptional activity producing Cyclin D, Cyclin E, Myc and EFG factors, all of which lead to carcinongenesis. In the absence of Hedgehog ligand, PATCH associates with smo, effectively blocking smo function and ultimately inhibiting carcinogenesis, thus suggesting the utility of Hedgehog inhibitors such as cyclopamine in breast cancer treatment.

The Notch pathway has been implicated in normal cell proliferation control and apoptosis as well as the development of a variety of organs. This pathway has been demonstrated to be abnormally regulated in cancer stem cells, including BCSC, leading to uncontrolled BCSC self-renewal. The Notch receptors are bound by ligands called Delta-like and Jagged. The bound receptors are proteolytically cleaved by ADAM protease family and y-sectretase, allowing sequestration into the nucleus. Once in the nucleus, the transcription of genes inhibiting cell differentiation and increasing cell proliferation ensues. The released

intracellular domain of Notch acts as a transcriptional co-activator to promote transcription of downstream targets of the recombination signal sequence binding protein Jk such as Myc and Cyclin D1 (Figure 7). Additionally, Notch transmembrane receptors for Notch proteins Notch 1-4 have been found in many stem cells. Studies have shown that treatment of ductal carcinomas with Notch inhibitors have led to the formation of fewer mammospheres, further supporting evidence of Notch playing a key role in mammary epithelial cell proliferation and differentiation. In fact, studies nearly a decade ago provided evidence for a role of Notch in breast cancer. These studies showed hyperproliferation of normally mammary cells in a dose-dependent manner by Notch pathway activation of constitutively active Notch receptors. Notch inhibitors are being considered in clinical trials also because Notch signaling has been implicated in breast cancer to resistance to radiation therapy.

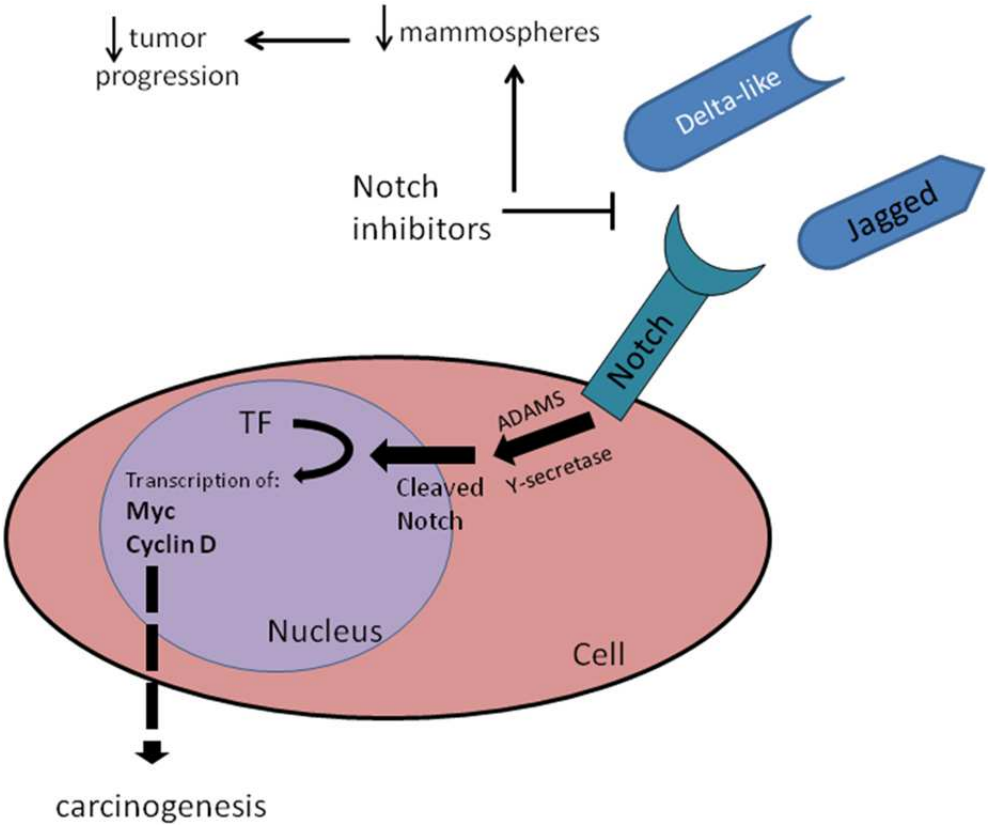

Fig. 7. Role of Notch in breast cancer.
The intracellular domain of Notch is cleaved by ADAMS and y –secretase after being bound by Delta-like and Jagged ligands. The cleaved portions are then sequestered in the nucleus where they act as transcription cofactors resulting in increased Myc and Cyclin D, promoting carcinogenesis. Also, Notch inhibitors have been demonstrated to decrease mammosphere formation, further suggesting the importance of Notch in breast cancer therapy.

5. Treatments

Since the discovery of stem cells in breast cancer, these cells have also been the focus of research as potential targets for anti-neoplastic treatment. If these cells allow cancer progression, resistance, and recurrence, it is logical that treatments concentrated on stem cell suppression would lead to more efficacious breast cancer treatment. Importantly, long-term efficacy is doubtful since renewal of stem cells is always a possibility. Nonetheless, there are have many proposed therapies targeted at BCSC and their pathways. To date, several potential treatments have been suggested against the MDR profile of BCSC including UHRF1, dofequidor fumarate, isotetrandrine. UHRF1 plays a role in treating breast cancer by inhibiting MDR1 promoter activity and expression. Studies have shown that overexpression of UHRF1 can induce deacetylation of histonesH3 and H4 on the MDR1 promoter. This deacetylation leads to a loss of binding to transcription factors MyoD, CBP, and p300, ultimately suppression MDR1. These cells have been shown to have increased sensitivity to chemotherapy agents that are transported by p-glycoprotein. Dofequidor fumarate, an orally active quinilone compound, has been shown to decrease MDR profile by inhibiting p-glygoprotein, MDR1 or both. Indeed, phase III clinical trials have shown a decrease in chemo-resistance in patients who have not previously received treatment. The compound is thought to work by inhibiting ABCG2/BCRP, which is higher in side populations than non side population cells, which results in an increased sensitivity to anti-neoplastic treatment. Another agent found to be efficacious against the MDR profile associated with BCSC is isotetrandrine, an isoquinoline alkaloid extracted from Caulis manhoniae. This agent has been discovered to result in MDR reversal through inhibition of p-glycoprotein-mediated MDR as has phenybutenoids derived from the rhizomes of Zingiber cassumunar, both acting as a potent chemo-sensitizing agent in the treatment against breast cancer. Additionally, IL-24 has recently gained attention as a potential anti-neoplastic treatment based on its anti-tumor effects via induction of apoptosis. Other anti-BCSC agents include salinomycin, an agent that targets cancer stem cells of epithelial origin. This agent has been shown to decrease the population of CD44+/CD24-/low within breast cancer. Although not specifically shown to inhibit breast cancer progression, cyclopamine has been demonstrated to inhibit progression of other cancers through the Hedgehog pathway, which is responsible for maintenance of CD44+/CD24-/low population in breast cancer.

Since de-differentiation is a major focus as a potential origin of BCSC and the de-differentiated phenotype is associated with aggressive breast cancers, it follows that agents that can induce differentiation could serve as possible agents antagonistic to stem cell creation and survival, leading to improved prognosis and treatment efficacy. Retinoids are the forerunners for differentiation therapy and has been shown to be successful as seen in acute myeloid leukemia M3 therapy where all-trans retinoic acid is currently being used. Of note, BRCA1 has been shown to be integral in differentiation in breast cancer. BRCA1 knockdown is present in breast cancers with increased mammosphere formation, increased ALDH1 cells and increased BCSC.

It is imperative also to consider the interaction of the immune system with breast cancer cells prior to initiating targeted treatment against BCSC. The helper T cell phenotype (Th1 phenotype), has been found to be more anti-neoplastic than the counterbalancing Th2 phenotype. Th1 phenotype have effects that may be bimodal, however, since the inflammatory nature of the Th1 response can cause DNA damage leading to malignant cell transformation, while also allowing anti-tumorigenic actions, perhaps to eliminate these

malignant cells, as seen by micrometastases to sentinel lymph nodes creating an increased Th1 phenotype. The Th2 phenotype, which studies show to be more clearly linked to pro-tumorigenic processes, demonstrates a facilitative effect on cancer via release of Il-4, which prevents chemo-sensitivity and escape from immune detection.

Pathways associated with BCSC have also been targeted in breast cancer research. There is significant evidence of a role for Hedgehog and Wnt, on stem cells in breast cancer, however, there is difficulties associated with inhibiting these pathways because they are also involved in normal somatic stem cells required in development. Additionally, since Numb, an inhibitor of the Notch pathway, has been shown to have decreased activity in BCSC, targeted therapies against Notch have made it to clinical trials unfortunately however, with limited success. Much of the ineffectuality of the treatments has been attributed to potential cross talk with other pathways.

6. Conclusion

Breast cancer research has come a long way in uncovering the unique characteristics of stem cells found within breast cancers that make them an important aspect in breast cancers as both a reason for resistance and progression as well as a potential target for anti-neoplastic treatment. Although much has been discovered about BCSC since their introduction into breast cancer research back in 1997, there is still a lot to be figured out. There have been a lot of characteristic that have been found helping identify BCSC, however, there is still a lack of a concrete definition. Indeed, it may be not only unrealistic to determine a single definition of BCSC, but also counterproductive. Since there are such a variety of breast cancers and treatments vary accordingly, maybe it is only natural that the definition of these BCSC would also vary. Even after stem cells have been identified, it is difficult to specifically target these cells. Given that BCSC, whether or not they result directly from normal stem cells, have a lot of pathways common to both the abnormal and normal stem cells. This makes it difficult to inhibit these pathways without altering normal cellular processes necessary for normal cell survival as well. Determination of the development and origin of BCSC would be greatly helpful in establishing specific treatments focused on these malignant cells. Given the plethora of information already discovered about BCSC and the vast areas still being investigated, research has shown how critical BCSC are in the fight against breast cancer.

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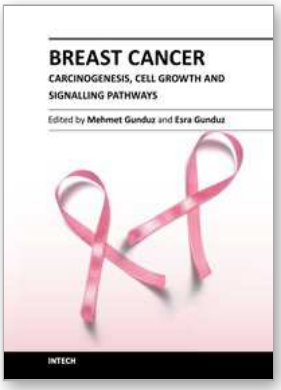

Breast Cancer - Carcinogenesis, Cell Growth and Signalling Pathways

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Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed various aspects of breast cancer carcinogenesis from clinics to its hormone-based as well as genetic-based etiologies for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

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