

Perspective

# Breast Tumor Heterogeneity

## Cancer Stem Cells or Clonal Evolution?

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### ABSTRACT

Breast tumors are composed of a variety of cell types with distinct morphologies and behaviors. It is not clear how this tumor heterogeneity comes about. Two popular concepts that attempt to explain this are the cancer stem cell hypothesis and the clonal evolution model. Each of these ideas has been investigated for some time, leading to the accumulation of numerous findings that are used to support one or the other. Although the two views share some similarities, they are fundamentally different notions with very different clinical implications. Analysis of the research backing each concept, along with a review of the results of our recent study investigating putative breast cancer stem cells, suggests how the cancer stem cell hypothesis and the clonal evolution model may be involved in generating breast tumor heterogeneity. An understanding of this process will allow the development of more effective ways to treat and prevent breast cancer.

### ABBREVIATIONS

NOD/SCID, nonobese diabetic-severe combined immunodeficiency; FISH, fluorescence in situ hybridization; CGH, comparative genomic hybridization; SAGE, serial analysis of gene expression; SNP, single nucleotide polymorphism.

### INTRODUCTION

Breast cancer and other epithelial tumors, or carcinomas, are the most prevalent malignancies in humans.<sup>1</sup> Breast tumors may be classified by how much they have spread to other tissues as in situ, invasive, or metastatic lesions.<sup>2-3</sup> In addition, research has shown that there are multiple subtypes of breast tumors, each with their own gene expression patterns and associated clinical outcomes.<sup>4</sup> Each case of breast cancer is thought to originate from a single cell that accumulates multiple mutations, allowing it to ignore normal growth controls and proliferate indefinitely.<sup>5-7</sup> In the past decade, earlier detection and improved treatments have reduced breast cancer mortality rates; however, incidence and recurrence rates for the disease remain high,<sup>8-9</sup> and therapy is usually not personalized and can have many detrimental side effects. These problems exist largely because the precise biological mechanisms that underlie breast cancer initiation and progression remain poorly understood.

One characteristic that all cancers and tumor types have in common is a striking variability among the cancer cells within a single tumor.<sup>10</sup> These cells differ in features such as size, morphology, antigen expression, and membrane composition as well as behaviors such as proliferation rate, cell-cell interaction, metastatic proclivity, and sensitivity to chemotherapy.<sup>11-12</sup> This “tumor heterogeneity” complicates the study and treatment of cancer since tumor samples may not be representative of the whole<sup>13</sup> and because its origins are unclear. On the positive side, tumor heterogeneity is a sort of written history of a particular cancer from which we can learn. Determining the molecular events that control this tumor trait would be a major breakthrough in our knowledge of cancer development and could lead to more effective breast cancer therapies and prevention methods.

Two current ideas that attempt to describe the establishment and maintenance of tumor heterogeneity are the cancer stem cell hypothesis and the clonal evolution model. However, as of May 5, 2007, a PubMed search for the terms “clonal evolution” and “cancer stem cells” returns no results. Thus, there is a marked lack in the scientific literature of a

critical comparison of these two important cancer biology concepts. This article therefore presents the evidence supporting each idea, their similarities and differences, and how each may contribute to breast tumor heterogeneity based on our recent findings and the work of others.

## THE CANCER STEM CELL HYPOTHESIS

**Description of the concept.** The cancer stem cell hypothesis states that a particular subset of tumor cells with stem cell-like properties, called “cancer stem cells,” drive tumor initiation, progression, and recurrence. By definition, these cells have the abilities to self-renew indefinitely and to differentiate, characteristics of normal adult stem cells. Their self-renewal and differentiation lead to the production of all cell types of a tumor, thereby generating tumor heterogeneity. Meanwhile, the other cells in a tumor do not have unlimited self-renewal capacity and cannot differentiate to produce all tumor cell types. Cancer stem cells are widely believed to arise from normal stem or progenitor cells of an organ and are thought to persist as a small fraction of the cells in a tumor. Also, according to the cancer stem cell hypothesis, tumor progression is a result of the metastatic spread of these cells, and cancer recurrence is caused by their resistance to therapy.<sup>10,14-26</sup>

**Supporting evidence.** The idea that cancers originate from rare stem cells is a long-standing one, first proposed over a century ago by Cohnheim,<sup>27</sup> that is supported by many general observations of biology. First of all, normal stem cells may be the cells in which cancer begins. These have been shown to exist in many tissues from which cancer often develops, such as blood, brain, lung, and prostate.<sup>28-31</sup> They are also long-lived, making them more likely than other cells to acquire the multiple mutations needed to become cancer.<sup>32</sup> Second, the tumor traits of monoclonality, unlimited proliferative capacity, and phenotypic heterogeneity that includes a variety of differentiation states with some non-dividing cells<sup>14</sup> could be explained by tumors originating from a self-renewing, multipotent, and slow-cycling cell. Third, normal stem cells and cancer cells are both believed to be regulated by epigenetic mechanisms and their microenvironments, and they share many abilities, including induction of angiogenesis, resistance to apoptosis and drugs, and cell migration, implicating stem cell-like cancer cells in initiation, recurrence, and metastasis.<sup>22-23,32-36</sup> In addition, cancer cells often have pathways associated with stem cell function, such as Wnt, Hedgehog, and Notch, either activated or deregulated.<sup>15,19</sup> Fourth, some other results show that normal stem cell function may play a role in metastasis. For example, the chemokine receptor CXCR4, which is found on normal hematopoietic stem cells as well as on many cancer cells, promotes metastasis in a variety of tumors.<sup>22</sup> Also, metastases of most carcinomas recapitulate the organization of their primary tumors.<sup>1</sup> Finally, the observed requirement of a large number of tumor cells to transplant tumors, even between syngeneic mice with identical immune systems, suggests that only a fraction of tumor cells are tumorigenic.<sup>19,22</sup>

Much evidence supporting the cancer stem cell hypothesis has been generated recently. Cells with properties of cancer stem cells were first identified in 1994 when researchers showed that a small population of cells from human acute myeloid leukemia that expressed some cell surface markers associated with normal hematopoietic stem cells could initiate leukemia in NOD/SCID mice while

other isolated cells could not.<sup>37</sup> Since then, this assay has become the accepted standard method for determining whether cell populations isolated from solid tumors are “cancer stem cells” or not.<sup>20-21,26</sup> Cells are purified from tumor samples based on their cell surface marker expression, ability to form spheres in culture, or exclusion of the dye Hoechst 33342 and are then tested for the ability to form tumors when injected into immunodeficient mice. Cancer stem cells have been reported in many tumor types such as lung, brain, skin, prostate, and colon.<sup>30,38-41</sup>

**Study in breast cancer.** The existence of stem cells in the mammary gland has long been postulated since this organ undergoes morphological changes throughout life, particularly during and after pregnancy.<sup>24</sup> Recent *in vitro* studies have identified and cultured breast cells capable of giving rise to both luminal epithelial and myoepithelial cells.<sup>42-45</sup> Furthermore, a functional mouse mammary gland has been generated from a single cell.<sup>46-47</sup> These demonstrations that normal breast stem cells exist indicate that stem cells could play a role in breast cancer. This is further suggested by the finding that early first full-term pregnancy is associated with lower lifetime breast cancer risk.<sup>48</sup> This might be explained if transformation preferentially occurs in normal mammary stem cells since these may be present in fewer numbers once the differentiation associated with pregnancy has occurred.<sup>49</sup> In addition, most breast cancers arise within a relatively short segment of terminal ductules that might be the location of normal stem cells.<sup>18</sup> Finally, a marker associated with normal breast stem cells, CD44, was used to identify breast cancer stem cells in 2003. These were the first solid tumor cancer stem cells to be isolated. In particular, Al-Hajj and colleagues<sup>50</sup> showed that human breast cancer cells that have a CD44<sup>+</sup>CD24<sup>-/low</sup> phenotype and do not express various normal cell markers could efficiently form tumors containing an array of cell types similar to those found in the original carcinoma samples when injected into immunocompromised mice, while CD44<sup>+</sup>CD24<sup>+</sup> cancer cells could not. Reinforcing these findings, others have shown that breast and prostate cancer cells that express CD44 are tumorigenic and have progenitor-like properties.<sup>40,51</sup>

## THE CLONAL EVOLUTION MODEL

**Description of the concept.** The clonal evolution model of carcinogenesis states that cancer cells over time acquire various combinations of mutations within a tumor and that genetic drift and stepwise natural selection for the fittest, most aggressive cells drive tumor progression. According to this idea, tumor initiation takes place once multiple mutations occur in a random single cell, providing it with a selective growth advantage over adjacent normal cells. As the tumor progresses, genetic instability and uncontrolled proliferation allow the production of cells with additional mutations and hence new characteristics. These cells may leave a large number of offspring by chance, or the new mutations may provide a growth advantage over other tumor cells such as resistance to apoptosis. In either case, primarily the latter, new subpopulations of variant cells are born, and other subpopulations may contract, resulting in tumor heterogeneity. Through this process, which occurs throughout the lifetime of a tumor, any cancer cell can potentially become invasive and cause metastasis or become resistant to therapies and cause recurrence.<sup>13,25,52-56</sup>

**Supporting evidence.** Nowell first proposed the clonal evolution model in 1976.<sup>52</sup> He observed that tumors generally seem to lose properties of differentiation as they progress and interpreted this removal of specialized functions as a way for them to maximize their proliferation and invasiveness. Such a task could be accomplished by selective pressures within a tumor. The idea of clonal evolution is supported by several other general cancer phenomena. One of these is the instability of cancer cell genomes, a feature broadly recognized as an “enabling characteristic” of cancer.<sup>6</sup> Other tumor traits can be accounted for by clonal evolution, including their monoclonality, unlimited proliferative capacity, heterogeneous make-up with cells that have various morphologies and metabolic differences, and interplay with the microenvironment. These observations could be explained by tumors originating from a single cell and undergoing continuous alterations that select for the most fit cells under local conditions.<sup>13</sup> Clonal evolution is also supported by findings that various drug-resistant clones are observed after some cancer therapies, such as treatment with the alkylating agent temozolomide or the tyrosine kinase inhibitor imatinib.<sup>57-58</sup> Finally, several studies of various cancers have shown that the patterns of genetic alterations seen between primary tumors, metastases, and recurrences match what is expected from clonal evolution. Within individual patients, most mutations in primary tumors and metastases are identical, but some are unique to each; increasing degree of tumor cell chromosomal aberration correlates with more malignant properties; and recurrences contain the mutations seen before in primary tumors along with additional ones.<sup>53,59-60</sup> Studies of pre-malignant lesions such as colorectal adenomas have similarly found that more mutations tend to occur in more advanced neoplasms,<sup>61</sup> indicating that clonal evolution likely occurs during tumorigenesis and may therefore continue during tumor progression.

Perhaps the most convincing evidence for clonal evolution comes from studies of cancer cells taken from various regions within a single tumor using laser-microdissection. Such work reveals that every major type of human cancer and histological subtype contains at least several cell subpopulations with differing heritable abnormalities.<sup>11,13,23</sup> The separation of these clones in distinct areas of a tumor occurs because sister cells usually remain contiguous in solid tumors.<sup>11</sup> Examples of mutational heterogeneity that have been found are diploid and aneuploid clones within a tumor<sup>53</sup> and different allelic losses in cells from the same tumor.<sup>62</sup> Genetic differences are also observed between cells in different regions of individual pre-malignant lesions. For instance, this has been seen in Barrett’s esophagus, a condition associated with increased risk of developing esophageal adenocarcinoma.<sup>63</sup>

**Study in breast cancer.** In breast carcinomas, patterns of mutation have been shown to reflect the process of clonal evolution. For example, in situ and invasive breast cancer cells from the same patient are generally identical genetically but can have different allelic losses.<sup>64</sup> Similarly, matched primary breast tumors and metastases share most genetic changes but can also have additional ones.<sup>65</sup> Also, multiple research groups have shown that individual breast tumors contain significant mutational heterogeneity.<sup>66</sup> For example, using FISH, Aubele and colleagues<sup>67</sup> found that different cells from the same invasive breast tumor had different chromosomal gains and losses. Likewise, Torres and others<sup>66</sup> showed that primary breast carcinomas are composed of several genetically heterogeneous and spatially separated cell populations using CGH, and Fujii and

coworkers<sup>64</sup> identified distinct allelic losses in different cells from the same in situ breast cancer. In particular, the copy numbers of *ERBB2* and other genes have been shown to vary within a breast tumor.<sup>68-69</sup>

## CANCER STEM CELLS VS. CLONAL EVOLUTION

**Similarities.** There are a few points on which the cancer stem cell hypothesis and the clonal evolution model agree. In each case, tumors originate from a single cell that has acquired multiple mutations and has gained unlimited proliferative potential. Also in each case, the specific cell of origin, particular genetic alterations involved, and microenvironmental factors can influence the make-up of a tumor and determine its physical characteristics and clinical properties. In addition, both the cancer stem cell hypothesis and the clonal evolution model may involve tumorigenic cells with stem cell-like properties since these characteristics may be either traits of the tumor cells of origin or impose a selective growth advantage over other tumor cells.

**Differences.** The cancer stem cell hypothesis and the clonal evolution model differ in several key ways (Fig. 1). First of all, they explain tumor heterogeneity with different mechanisms: either a program of aberrant differentiation or a competition among neighbors primarily produces the multitude of cell types seen in a tumor. Second, normal stem and progenitor cells are considered the most likely targets of transformation according to the cancer stem cell hypothesis, while no normal cells in particular are identified as such by the clonal evolution model. Third, the cancer stem cell hypothesis states that only a small pool of cells, the “cancer stem cells,” contribute to tumor progression, while the clonal evolution model supposes that any tumor cell has the potential to be involved in this process. Related to this concept, in the cancer stem cell hypothesis, only cancer stem cells may further mutate and thereby possibly become more aggressive, while in the clonal evolution model, mutations may occur in any tumor cell. Following from this, the two ideas allow for different cell populations to have the ability to self-renew: either only cancer stem cells or any cells that acquire this capacity. Another difference between the two theories is the way that each explains therapeutic resistance: either cancer stem cells are inherently drug-resistant or therapy selects for tolerant clones.

**Therapeutic implications.** Distinct therapeutic strategies are suggested by the cancer stem cell hypothesis and the clonal evolution model. According to the cancer stem cell hypothesis, killing the rare cancer stem cells that fuel tumor growth will cause tumors to fall apart and cure disease. In contrast, according to the clonal evolution model, treatment must result in death of the multiple cancer cell populations that contribute to tumor progression to be curative. Targeting cancer stem cells requires knowledge of proteins and pathways that are specifically expressed in or required for these cells and not normal stem cells. Based on this information, therapies that could be designed to eliminate cancer stem cells include antibody treatment, pharmacological agents, and differentiation therapy.<sup>10,21-22</sup> Similar methods could be used to target cell populations that have resulted from clonal evolution, but multiple drugs would likely need to be used together. Since resistance mutations may occur partway through tumor progression in the clonal evolution model, early intervention may be an especially important facet of treatment if this idea is correct.<sup>11,13</sup>

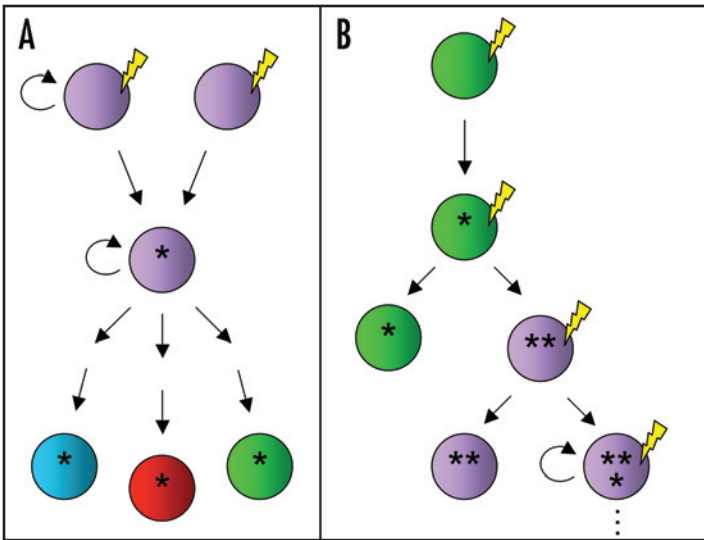


Figure 1. Two proposed explanations of cancer initiation and progression. (A) According to the cancer stem cell hypothesis, cancer-causing mutations likely occur in normal adult stem cells, which are self-renewing, or progenitor cells. These cells give rise to “cancer stem cells” that can self-renew and also differentiate to produce all the other cells of a tumor. These progeny are not able to self-renew and are genetically identical to the cancer stem cells. Although not indicated here, the cancer stem cells might further mutate. (B) In the clonal evolution model, any normal cell (such as a differentiated cell, as shown here) may be the target of transformation. Then, a cancer cell may acquire an additional mutation and give rise to a new line of tumor cells, while some cancer cells without the latest mutation remain. This process can repeat ad infinitum, and some mutations may confer new traits such as dedifferentiation or self-renewal ability. Cells with mutations that give some growth advantage over others will dominate the tumor. In both (A) and (B), circles represent cells (with purple indicating stem or progenitor properties), lightning bolts represent mutagenesis, and stars represent mutations. The first star in each circle stands for the multiple mutations needed to convert a normal cell to a cancer cell.

## EVIDENCE IN FAVOR OF ONE CONCEPT

**Our recent study of putative breast cancer stem cells.** To investigate the mechanisms that may control the differences between putative breast cancer stem cells and more differentiated breast cancer cells, our lab recently performed a comprehensive molecular characterization of these two classes of cells. Using the markers previously associated with breast cancer stem cells by Al-Hajj and colleagues,<sup>50</sup> we isolated CD44<sup>+</sup>CD24<sup>-</sup> (“CD44<sup>+</sup>”) cells and more differentiated CD44<sup>-</sup>CD24<sup>+</sup> (“CD24<sup>+</sup>”) cells from human breast tumor samples and generated SAGE libraries, which enumerate the gene transcripts found in each cell type. Our results, published in the March 2007 issue of *Cancer Cell*,<sup>70</sup> indicate that CD44<sup>+</sup> cells are indeed more stem cell-like and less differentiated than CD24<sup>+</sup> cells based on their expression of various stem and differentiated cell markers. They also show that various pathways, such as TGF $\beta$ , Wnt, and Hedgehog in CD44<sup>+</sup> cells, are activated in each cell type and that CD44<sup>+</sup> cells are enriched for the expression of genes involved in cell motility, angiogenesis, and invasion compared to CD24<sup>+</sup> cells. Gene signatures characteristic of CD44<sup>+</sup> and CD24<sup>+</sup> cells in primary tumor microarray data identified groups of patients with shorter and longer distant metastasis-free survival times, respectively. In addition, we determined the gene expression profiles of normal CD44<sup>+</sup> and

CD24<sup>+</sup> cells and discovered that cells of the same type (e.g., normal CD44<sup>+</sup> cells and cancer CD44<sup>+</sup> cells) appear more similar than cells of the same tissue (e.g., normal CD44<sup>+</sup> cells and normal CD24<sup>+</sup> cells). We saw an apparent decrease in cells expressing CD44<sup>+</sup> cell markers in normal breast tissue in late pregnancy and an increase in the frequency of cells expressing CD24<sup>+</sup> cell markers in distant metastases compared to in matched primary tumors. Surprisingly, we also found using SNP arrays and FISH that although CD44<sup>+</sup> and CD24<sup>+</sup> cells from the same tumor are clonally related, genetic differences can exist between the two populations. Overall, our results provide a portrait of the molecular differences between two distinct cell types that contribute to breast tumor heterogeneity and provide some clues as to whether cancer stem cells or clonal evolution is at work.

### How well our results support each tumor progression model.

Our findings support several aspects of the cancer stem cell hypothesis. First, putative breast cancer stem cells (cancer CD44<sup>+</sup> cells) may be derived from normal stem cells since they express many stem cell markers and are similar to normal CD44<sup>+</sup> cells, which may be normal breast stem cells. (They also express stem cell markers, and cells positive for CD44<sup>+</sup> cell markers seem to decrease in late pregnancy, as is expected for normal breast stem cells.) Second, cancer CD44<sup>+</sup> cells may give rise to a hierarchy of tumor cells resembling that in the normal mammary gland since cancer CD24<sup>+</sup> cells are similar to normal CD24<sup>+</sup> cells and have genetic alterations not present in cancer CD44<sup>+</sup> cells. Third, cancer CD44<sup>+</sup> cells have a more migratory, angiogenic, and invasive phenotype, indicating that these potential cancer stem cells could contribute to metastasis, which was also suggested by our finding that the presence of a cancer CD44<sup>+</sup> cell gene signature in primary tumors correlates with higher risk of distant metastasis.

Some of our findings also support the clonal evolution model, in part by contradicting portions of the cancer stem cell hypothesis. First, the finding that CD44<sup>+</sup> and CD24<sup>+</sup> cells within a tumor are clonally related but that the CD24<sup>+</sup> cells have an additional genetic alteration shows that CD24<sup>+</sup> cells can acquire mutations independently of CD44<sup>+</sup> cells. This observation is not compatible with the strictest interpretation of the cancer stem cell hypothesis since it indicates that CD44<sup>-</sup>CD24<sup>+</sup> cells, which were previously shown to be largely non-tumorigenic, could potentially contribute to breast cancer progression. It is conceivable that these cells could undergo a mutation that makes them more tumorigenic or otherwise cancer-promoting. In contrast, our finding fits the clonal evolution model well. Second, the detection of gene signatures characteristic of cancer CD44<sup>+</sup> and CD24<sup>+</sup> cells in primary bulk tumor gene expression data indicates that there is probably a large portion of each cell type in most tumors. Therefore, CD44<sup>+</sup>CD24<sup>low</sup> breast “cancer stem cells” are likely not a small fraction of tumor cells as postulated by the cancer stem cell hypothesis. However, they and CD44<sup>-</sup>CD24<sup>+</sup> cells may be cancer cell subpopulations competing for dominance as in the clonal evolution model. Finally, our observation that metastases contain a higher frequency of CD24<sup>+</sup> cells than do matched primary tumors may support clonal evolution since it could mean that cancer CD24<sup>+</sup> cells thrive in the different environments they encounter at sites of metastasis.

**Concerns about studies identifying cancer stem cells.** The methods used to identify cancer stem cells from solid tumors may not accurately do so, questioning the validity of the cancer stem

cell hypothesis. In order to isolate subpopulations of cells, tumor samples are usually treated with proteolytic enzymes and then sorted by staining with antibodies or Hoechst 33342 followed by flow cytometry or by purification with immunomagnetic beads. These approaches can harm certain cells and thereby artificially select for others.<sup>19,26</sup> Also, cell recovery from solid tumors is usually 10% or less, so samples analyzed may not be representative of the original lesions.<sup>19</sup> Therefore, actual cancer stem cells may be missed, while other cells may be inaccurately identified as cancer stem cells. The determination of whether or not isolated cell populations are tumorigenic by injecting them into immunodeficient mice may not reflect the behavior of cells in patients for many reasons. Of course, studies in mouse are usually not comparable to what is happening in humans due to the differences between the two species. In particular, injecting cells into mice does not mimic the numerous effects that the human cancer cell microenvironment is believed to have on solid tumor initiation and progression.<sup>1,11-12,17-18,35,53,71</sup> Furthermore, since the mice used are immunocompromised, effects of an immune system are not measured. Additionally, the efficiency of tumor transplantation in mice and other animal models depends on the location of transplantation.<sup>72-74</sup> Therefore, this assay really measures how well some cells can grow in a particular mouse strain when injected in a certain way. Also, injecting isolated populations of cells does not recapitulate the interactions between tumor cells, which can affect growth rate, metastatic ability, and other behaviors.<sup>19,75</sup> The assay is essentially selecting for cells that can survive on their own, are angiogenic, and can engraft well, properties that they might not necessarily need to have to initiate tumors in humans.<sup>21</sup> This means that identified “cancer stem cells” may not be the true or only cells in human patients that are tumorigenic and accordingly may not represent only a small percentage of tumor cells; thus, the main assertion of the cancer stem cell hypothesis that a rare population of cells in a tumor are carcinogenic may be incorrect. Indeed, recent evidence has shown that all cells in the C6 glioma cell line have cancer stem cell properties but only under certain conditions.<sup>26,76</sup>

## POSSIBLE MODEL AND FINAL REMARKS

**Conclusions.** Based on the information presented here, it seems that breast tumor heterogeneity is likely caused by a version of the clonal evolution model that incorporates some features of the cancer stem cell hypothesis (Fig. 2). Tumor initiation may take place in a normal mammary stem or progenitor cell that may express CD44. The resulting cancer cell may self-renew and undergo a combination of differentiation and clonal selection<sup>15</sup> driven by the microenvironment and mutations, producing a variety of genetically and developmentally distinct tumor cells, including some that express CD24. Some differentiated cells may have less proliferative potential, while some mutated cells may acquire self-renewal capacity, a higher proliferation rate, and other cancer-promoting traits. Due to differences between tumor locations, individuals, and the specific mutations that occur, cells with different molecular properties may end up driving different breast tumors. Ultimately, tumors may have different percentages and varieties of cells expressing CD44 and CD24, which could explain why both of these markers have been associated with metastasis.<sup>77-78</sup>

**Significance.** Determining whether the cancer stem cell hypothesis or the clonal evolution model is a more accurate description

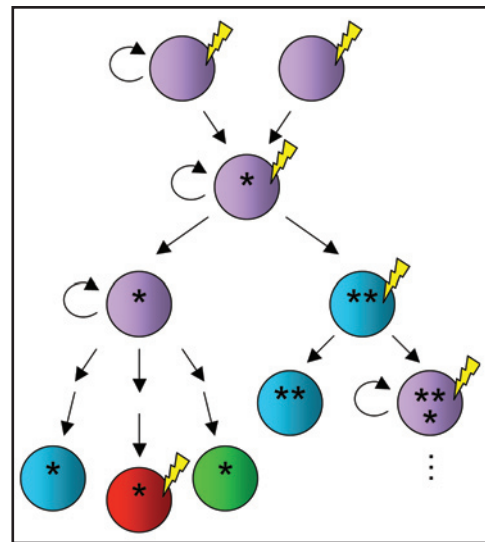


Figure 2. A view of breast cancer development based on the clonal evolution model and including some features of the cancer stem cell hypothesis. In this model, CD44<sup>+</sup> normal mammary stem or progenitor cells acquire cancer-causing mutations. This results in CD44<sup>+</sup> cancer cells that can self-renew and differentiate into all tumor cell types, including CD24<sup>+</sup> cancer cells. Additional mutations may occur in any of these cells, leading to differentiation, dedifferentiation, or the acquisition of new characteristics. For instance, as indicated here, a CD44<sup>+</sup> cancer cell may mutate to become more differentiated and then again to acquire self-renewal ability and more stem cell-like traits. In addition, a CD24<sup>+</sup> cancer cell may become further mutated. Cells with growth advantages over others will dominate the tumor. Although not shown here, the continued mutation and selection of cell populations may lead to cancer cells that can self-renew and differentiate into all tumor cell types but do not express CD44. Circles represent cells (with purple and red indicating CD44 and CD24 expression, respectively), lightning bolts represent mutagenesis, and stars represent mutations. The first star in each circle stands for the multiple mutations needed to convert a normal cell to a cancer cell.

of cancer progression is critical for the advancement of research in this field and for the development of effective cancer therapies. The cancer stem cell hypothesis has generated much enthusiasm recently and is therefore the focus of much study; however, there are still questions concerning its actual role in cancer. A deeper understanding of its relationship with the clonal evolution model may guide future research. Treatments that target cancer stem cells have been proposed as alternatives to current cancer therapies. Importantly, the possible role of clonal evolution in cancer suggests that multiple tumor cell populations may need to be targeted by treatments for these to be successful. For instance, in breast cancer, a combination of drugs directed against cancer CD44<sup>+</sup> and CD24<sup>+</sup> cells may be most effective. Treatments that target only cancer CD44<sup>+</sup> cells and do not eradicate cancer CD24<sup>+</sup> cells may leave behind many dangerous metastatic cells.

**Future directions.** A better understanding of breast tumor heterogeneity will be obtained by the continued careful analysis of normal and tumor cell subpopulations. Determining the structure of the normal mammary cell hierarchy may provide clues as to how breast cells behave in tumors. Further purification and molecular profiling of tumor cell types will be useful for identifying possible ways of specifically targeting each. In addition, clinical trials that track particular cancer cells as tumors form and respond to treat-

ment are needed to test how these cells differentiate and evolve in patients.<sup>13</sup> Currently, the earliest stages of tumorigenesis cannot be readily detected.<sup>23</sup> However, once the mechanisms involved in breast cancer development are understood, we may be able to design ways to prevent the disease from occurring.

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