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Implications of the Cancer Stem-Cell Hypothesis for Breast Cancer Prevention and Therapy

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Abstract

Recent research in breast biology has provided support for the cancer stem-cell hypothesis. Two important components of this hypothesis are that tumors originate in mammary stem or progenitor cells as a result of dysregulation of the normally tightly regulated process of self-renewal. As a result, tumors contain and are driven by a cellular subcomponent that retains key stem-cell properties including self-renewal, which drives tumorigenesis and differentiation that contributes to cellular heterogeneity. Advances in stem-cell technology have led to the identification of stem cells in normal and malignant breast tissue. The study of these stem cells has helped to elucidate the origin of the molecular complexity of human breast cancer. The cancer stem-cell hypothesis has important implications for early detection, prevention, and treatment of breast cancer. Both hereditary and sporadic breast cancers may develop through dysregulation of stem-cell self-renewal pathways. These aberrant stem cells may provide targets for the development of cancer prevention strategies. Furthermore, because breast cancer stem cells may be highly resistant to radiation and chemotherapy, the development of more effective therapies for this disease may require the effective targeting of this cell population.

Introduction

There is both good news and bad news in the fight against breast cancer. The good news is that there has been a steady decline in the death rate from breast cancer in this country and abroad since 1990. A 2% annual decrease in the death rate has resulted in an overall 25% reduction in cancer deaths in 2007 compared with 1990.[1] Furthermore, the development of new treatments, such as trastuzumab and the aromatase inhibitors, has offered new hope to women with both early and advanced breast cancer. However, despite these clinical advances, as well

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as advances in our understanding of the biology of breast cancer, more than 44,000 women still die as a result of breast cancer annually in the United States alone. Recent analysis of the fall in death rates from breast cancer indicates that approximately half of this is the result of improved early detection through mammography screening, and the other half a result of improvements in adjuvant therapies for early-stage disease.[2] In contrast, there has been relatively little change in the overall survival for women with metastatic breast cancer during the last several decades.[3] Furthermore, even though recurrence rates have been significantly reduced by adjuvant therapies utilizing chemotherapy, hormonal therapy, and most recently, trastuzumab, an inhibitor of human epidermal growth factor receptor 2 (HER-2), recurrence still occurs in a substantial proportion of women after these treatments.

The heterogeneity and molecular complexity of breast cancer poses many challenges for the development of effective strategies to prevent and treat this disease. In addition, there is increasing support for the cancer stem-cell hypothesis, which, if correct, provides an explanation for the limitation of many current breast cancer models and suggests new strategies for breast cancer prevention and therapy. Classical models of carcinogenesis can be described as “stochastic” or “random,” in which any cell in an organ, such as the breast, can be transformed by the right combination of mutations.[4] As a result, all or most of the cells in a fully developed cancer are equally malignant. It follows that strategies designed to treat and ultimately cure these cancers require the killing of all these malignant cells. The cancer stem-cell hypothesis is a fundamentally different model composed of two separate, but interrelated, components. The first is that tumors originate in tissue stem and/or progenitor cells through the dysregulation of the normally tightly regulated process of self-renewal.[5] As a consequence, tumors contain a cellular component that retains key stem-cell properties including self-renewal, which initiates and drives carcinogenesis and differentiation, albeit aberrant, that contributes to tumor cellular heterogeneity.[6] Although the concept that cancers arise from germ cells or stem cells was first proposed more than 150 years ago,[7] it is only recently that advances in stem-cell biology have allowed for a more direct testing of the cancer stem-cell hypothesis. We will review recent evidence supporting this hypothesis and discuss its implications for breast carcinogenesis, cancer prevention, and cancer therapy.

Identification of Normal Breast Stem Cells

Stem cells are defined by their ability to undergo self-renewal, as well as multilineage differentiation. Self-renewal may be either symmetric, in which a stem cell produces two daughter stem cells, or asymmetric, in which the stem cell produces a daughter stem cell as well as a cell that leaves the stem-cell niche to differentiate.[8] In the mammary gland, these differentiating cells generate three lineages: ductal epithelial cells, which line ducts; alveolar epithelial cells, which are the milk-producing cells; and myoepithelial cells, which are contractile cells lining ducts and alveoli. Until recently, the isolation and characterization of breast stem cells was limited by the lack of identified cell-surface markers for these cells. The existence of stem cells in rodent mammary glands was first demonstrated by Kordon et al,[9] who showed the ability to repopulate mouse mammary glands with serial transplantation of retrovirally marked epithelial fragments. Similar mammary fat pad transplantation models have more recently been used to prospectively identify mouse mammary cells with stem-cell properties. Cells expressing CD29 and/or CD49F (β1 and α6 integrin, respectively) as well as CD24 displayed the stem-cell properties of self-renewal and multilineage differentiation.[10] A single cell from the $CD29^{high}/CD24^{+}$ or $DC49F^{high}/CD24^{+}$ population was able to reconstitute a functional mammary gland when this cell was transplanted into a cleared mouse mammary fat pad.[11] The murine mammary stem cell does not express estrogen receptor (ER) or progesterone receptor (ER) but is able to give rise to ER-expressing and PR-expressing cells.[12] Recently, our laboratory has provided evidence for the existence of similar stem cell–like populations in the human mammary gland characterized by expression of aldehyde

dehydrogenase 1. This enzyme has also been reported to be expressed in hematopoietic and neuronal stem and progenitor cells,[13,14] and can be detected utilizing an enzymatic "Aldefluor" assay or by immunohistochemistry utilizing antibodies to ALDH1.

Characterization of stem cells in both human and rodent systems has been facilitated by the development of in vitro culture systems that allow for propagation of mammary stem and progenitor cells in an undifferentiated state. Previously, it had been found that primitive neuronal cells could be propagated as floating spherical colonies termed "neurospheres."[15] On this basis, we hypothesized that normal and malignant stem cells might display anchorage independent growth, and utilized this property to develop a culture system for human mammary epithelial stem and progenitor cells. We demonstrated that such cells isolated from reduction mammoplasties when grown on nonadherent substrata in serum-free conditions in the presence of growth factors generate spherical colonies that we termed "mammospheres."[16] Mammosphere-initiating cells have stem-cell properties and are able to self-renew in vitro as well as differentiating into all three lineages found in the mammary gland. Furthermore, mammosphere-initiating cells express aldehyde dehydrogenase and are capable of generating human mammary structures when transplanted into the humanized fat pad of immunosuppressed nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice.[17]

Breast Cancer Stem Cells

Evidence for existence of cancer stem cells was first reported by Dick et al in acute myelogenous leukemia.[18] We utilized a similar approach to prospectively isolate similar populations of cells from human breast cancers. In collaboration with Michael Clarke, we demonstrated that human breast cancers contain a cellular population characterized by the expression of cell-surface markers $CD44^{+}/CD24^{low}/lin^{-}$, which display stem-cell properties. As few as 200 of these cells, comprising 1% to 10% of the total population, were capable of forming tumors when implanted in NOD/SCID mice.[19] In contrast, 20,000 cells that did not express these markers were unable to form tumors. Consistent with the cancer stem-cell model, the stem cells were able to generate tumors that recapitulated the phenotypic heterogeneity of the initial tumor.[19] As is the case with normal stem cells, breast tumor stem cells also form mammospheres in vitro.[20] Furthermore, as was the case with normal breast stem cells, breast cancer stem cells can be isolated on the basis of their increased expression of aldehyde dehydrogenase. Indeed, there is partial overlap between the $CD44^{+}/CD24^{-}/lin^{-}$ and ALDH-positive populations with cells expressing the phenotype $CD44^{+}CD24^{-}/lin^{-}$/ALDH-positive able to form tumors from as few as 20 cells.[17]

Clinical Implications of the Cancer Stem-Cell Hypothesis

The cancer stem-cell hypothesis has fundamental implications for breast carcinogenesis as well as important clinical implications for prevention and therapy. These are summarized in Figure 1.

Breast Carcinogenesis

The cancer stem-cell hypothesis proposes that cancers arise in breast, stem, and/or progenitor cells through dysregulation of the normally tightly regulated process of self-renewal. It is important to emphasize that this hypothesis does not require that all cancers arise from normal tissue stem cells. Indeed, there is substantial evidence in the hematopoietic system that leukemias may arise from transformed progenitor cells as well as stem cells. Overexpression of the *MLL* fusion gene in hematopoietic progenitors results in production of leukemias, which are driven by cells that acquire stem-cell properties.[21] Interestingly, these progenitors acquire the expression of self-renewal genes normally expressed in hematopoietic stem cells.[21] Recent

studies have demonstrated a similar phenomenon involving an ETS transcription factor, ETV6, fused to the protein tyrosine kinase domain of MTRK3, a molecular event that is found in human secretory breast carcinoma.[22] Li et al, reported that the *ETV6/NTRK3* fusion oncogene acts on committed mammary progenitor cells to produce breast cancers in transgenic mice.[22] Further evidence that stem/progenitor cells may be targets for transformation have been suggested by mouse mammary tumor virus (MMTV)-driven carcinogenesis models.[23] MMTV-Wnt tumors display markers of both epithelial and myoepithelial lineage, whereas MMTV Neu tumors show only luminal differentiation, suggesting that Wnt may effect a primitive bipotent progenitor cells, whereas NEU may target a luminal committed progenitor cell.[24] Furthermore, in MMTV-wnt-1 transgenic mice, the number of cells displaying stem-cell markers expanded more than six-fold in the preneoplastic phase, whereas MMTV Neu tumors did not demonstrate stem-cell expansion.[10] If similar events occur in human carcinogenesis, it could provide an explanation for aspects of molecular heterogeneity found in human breast cancers. Discrete molecular phenotypes revealed by gene expression analysis may reflect different cells of origin as well as the mutation profile in human breast cancers.[25]

Mammary Stem-Cell Number As a Determinate of Breast Cancer Risks

If breast tumors can originate in mammary stem cells through dysregulation of the self-renewal process, then breast stem-cell number may be a risk factor for carcinogenesis. Development of the mammary gland in humans and rodents is regulated at three critical periods of development: embryogenesis, puberty, and pregnancy. Changes in the hormonal milieu during these developmental windows may determine the size of the breast stem-cell pool, thereby influencing carcinogenesis.[26] Several studies have shown a strong link between birth weight and breast cancer risk in offspring, as well as a strong association of maternal levels of insulin-like growth factor-1 (IGF-1) and birth weight.[27] IGF-1 and steroid hormones in utero may modulate subsequent breast cancer risk by regulating the number of mammary stem cells.[28,29]

The growth hormone/IGF-1 axis may serve as a master regulator of adult stem cells in different organs.[30] Growth hormone, an anabolic pulsatile hormone secreted by the pituitary gland, is a major regulator of IFG-1 synthesis and secretion. Not only does growth hormone indirectly regulate cell proliferation mediated by IGF-1 but also acts directly on cells that express growth hormone receptor through stimulation of JAK/STAT (janus kinase/signal transducer and activator of transcription) signaling.[28] We have reported that mammary stem/ progenitor cells grown in mammospheres overexpress growth hormone receptors compared with cells induced to differentiate by attachment to a collagen substratum,[31] and have found that growth hormone stimulates mammary stem-cell self-renewal (unpublished data). Several clinical lines of evidence support a link between growth hormone levels and breast cancer risk. The rate of increase in height during adolescence, largely regulated by growth hormone, is strongly related to subsequent risk of breast cancer.[32] Furthermore, women with acromegaly who have increased growth hormone levels are at increased risk for developing cancers, including breast cancer.[30] The link between growth hormone and breast stem cells may also account for the development of aggressive breast cancers associated with pregnancy. Although pregnancy at an early age protects against subsequent breast cancer development,[30] breast cancers developing during pregnancy tend to be aggressive and of the basal phenotype, ER/PR/HER-2 negative.[33,34] Interestingly, high levels of estrogen and progesterone produced during the third trimester of pregnancy induce local growth hormone production by mammary epithelial cells. This suggests the intriguing possibility that the aggressive basal breast carcinomas associated with pregnancy may be driven by local production of growth hormone, which acts as a paracrine regulator of breast stem cells.[35]

Hereditary Breast Cancers and *BRCA1*

Heterozygous germline mutations in the *BRCA1* gene predispose women to up to an 80% lifetime risk of developing breast cancer.[35] Most of these tumors are of the basal phenotype, characterized by expression of myoepithelial markers, but lack expression of ER, PR, and ERBB2 receptor.[36] It is well established that *BRCA1* plays an important role in DNA repair, activation of cell-cycle checkpoints, and maintenance of chromosome stability. However, these characteristics do not explain the organ specificity of carcinogenesis. Foulkes et al[36] proposed that the clinical, molecular, and pathologic features of breast cancer in *BRCA1* mutation carriers suggest the possibility that *BRCA1* may function as a stem-cell regulator. The development of in vitro and mouse models for breast stem-cell function has allowed a direct test of this hypothesis. Utilizing these systems, we demonstrated that *BRCA1* expression is required for the differentiation of ER-negative stem/progenitor cells into ER-positive luminal cells.[37] Knockdown of *BRCA1* in primary breast epithelial cells leads to an increase in cells displaying the stem-cell marker ALDH1 and a decrease in cells expressing luminal epithelial markers and ER. Furthermore, in breast tissues from women with germline *BRCA1* mutations but not in normal controls, we detected entire lobules that, although histologically normal, were positive for ALDH1 expression but negative for expression of ER. Loss of heterozygosity for *BRCA1* was documented in these ALDH1-positive lobules but not in adjacent ALDH1-negative lobules.[37] These studies suggest that loss of BRCA1 expression may result in an accumulation of genetically unstable breast stem cells, providing targets for further carcinogenic events.

Self-Renewal Pathways in Breast Stem Cells

Elucidation of the pathways that regulate self-renewal of breast stem cells has led to a clearer picture of how dysregulation of these pathways may lead to carcinogenesis. Furthermore, these pathways may provide targets for breast cancer prevention and therapy. Indeed, recent evidence suggests that key oncogenic pathways known to be dysregulated in breast cancer also regulate stem-cell behavior.[26]

HER-2

An early event in the development of sporadic breast cancer may be the amplification and overexpression of the *HER-2* gene, a member of the epidermal growth factor receptor family. Approximately 20% to 25% of human breast cancers display *HER-2* amplification. These tumors have a distinct molecular profile and aggressive clinical course associated with the propensity to develop metastasis in areas such as the brain.[38] The development of *HER-2* inhibitors such as trastuzumab, or more recently, lapatinib, have provided important new agents with demonstrated clinical benefit in both adjuvant and advanced disease. The addition of trastuzumab to adjuvant chemotherapy reduces the recurrence rate by almost 50%.[39] Interestingly, in a series of 477 breast carcinomas, we found a significant correlation between expression of the stem-cell marker ALDH1 and *HER-2* overexpression.[17] Furthermore, we have recently found that *HER-2* overexpression in normal human mammary epithelial cells as well as mammary carcinomas increases the proportion of stem cells, as indicated by ALDH expression. Trastuzumab reduces the stem-cell population in trastuzumab-sensitive but not -resistant breast cancer cell lines.[40] Together, these results suggest that *HER-2* may play a role in mammary carcinogenesis by regulating the stem-cell population. If this is the case, then the remarkable clinical efficacy of *HER-2* inhibitors may be a result of the ability of these agents to directly target breast cancer stem cells.

PTEN

Another frequent abnormality in human breast cancers is deletion of the *PTEN* gene, a defect found in approximately 40% of human breast cancers.[41] Furthermore, women with *BRCA1*

germline mutations develop microdeletions of *PTEN*.[42] *PTEN* is lipid phosphatase which regulates phosphoinositide-3 kinase Akt signaling. *PTEN* has previously been shown to regulate self-renewal of hematopoietic and neuronal stem cells.[43] We have preliminary evidence that deletion of *PTEN* has similar effects on normal and malignant breast stem cells. This suggests that development of inhibitors of AKT or mammalian target of rapamycin signaling may be able to target normal and malignant breast stem cells.

Wnt Signaling

Wnt signaling has also been shown to be involved in regulating the self-renewal and differentiation of a variety of stem cells. As indicated previously, MMTV-Wnt mammary tumors display increases in cells expressing stem-cell markers.[10] Activation of the canonical Wnt pathway begins with the binding of Wnt proteins to cell-surface receptors in the Frizzled family and the low-density lipoprotein receptor–related proteins LRP5 and -6. This signaling increases cytoplasmic β-catenin, which translocates to the nucleus, where it binds to transcription factors in the LEF1/TCF family.[44] Wnt ligands have been shown to be expressed in embryonic mammary development[45] Embryos expressing the canonical Wnt inhibitor Dkk1 display a complete block formation of mammary placodes, and mice deficient for LEF1 failed to maintain mammary buds, demonstrating that Wnt signaling is required for normal embryonic mammary development.[46]

Notch Signaling

Other developmental signaling pathways have been shown to play a role in mammary carcinogenesis in murine models, as well as in human mammary cancer. Notch signaling has been shown to play a role in cell fate determination in neural, hematopoietic, and embryonic stem cells. In mammals, there are four notch receptors (notch 1-4) interacting with surface-bound or secreted ligands (Delta-like 1, Delta-like 3, Delta-like 4, Jagged 1, and Jagged 2).[47] Modifier proteins from the Fringe family (Lunatic, Manic, and Radical Fringe) modulate these interactions.[48] On ligand binding, Notch receptors are activated by serial cleavage events involving members of the ADAM protease family followed by intramembranous cleavage regulated by gamma secretase (presenilin). After proteolitic cleavage, the intracellular domain of Notch translocates to the nucleus to act on downstream targets such as the Hes and Hay transcription factors.[48] Approximately 40% of human breast cancers display reduced expression of the Notch inhibitor NUMB.[49] Interestingly, in addition to playing a role in the regulation of Notch signaling, NUMB may also regulate *p53*.[50] Evidence for the role of Notch signaling in mammary development has been provided by transgenic models. Dontu et al[51] demonstrated that Notch activation acts as a regulator of asymmetric cell fate decisions in human mammary cells by promoting mammary self renewal. In addition, Notch acts on later stages of mammary development affecting cell-fate commitment. Because the enzyme gamma secretase is necessary for Notch processing, gamma secretase inhibitors are able to inhibit Notch signaling.[52] Clinical trials utilizing gamma secretase inhibitors in combination with chemotherapy for women with advanced breast cancer are being initiated. Such trials will directly test the hypothesis that targeting breast cancer stem cells improves the therapeutic outcome in these women.

Hedgehog Signaling and *BMI-1*

Transgenic models have suggested a role for hedgehog signaling in normal mammary development and carcinogenesis. Furthermore, there is evidence for dysregulation of this pathway in a subset of human breast cancers. Utilizing both in vitro culture systems and NOD/SCID mice, Liu et al[53] demonstrated that hedgehog signaling regulates the self-renewal of both normal and malignant human mammary stem cells. Furthermore, this occurs by regulation of a polycomb gene *BMI-1*. This suggests that hedgehog signaling, acting through *BMI-1* is able

to regulate the self-renewal of normal and malignant human mammary stem cells. This process is blocked by specific inhibitors such as cyclopamine.[53] The development of cyclopamine analogs and other hedgehog inhibitors is currently underway, and clinical trials utilizing these agents are in the planning stages.

Mammary Carcinogenesis: A Conceptual Link Between Hereditary and Sporadic Breast Cancers

The elucidation of breast stem-cell self-renewal pathways suggests a conceptual link between hereditary and sporadic breast carcinogenesis. Both may be initiated by expansion of mammary stem and/or progenitor cells. In the case of hereditary breast cancer, this may occur via deletion of the normal allele of *BRCA1*. In sporadic breast cancers, activation of other pathways such as Notch, Hedgehog, or Wnt; amplification of *HER-2*; or deletion of *PTEN* may lead to dysregulation of stem-cell self-renewal, resulting in stem-cell expansion. These expanded stem cells provide targets for further carcinogenic events. This conceptual model is depicted in Figure 2. Clonal expansion of breast stem cells might explain the field carcinogenesis observed in human breast cancers. For instance, histologically normal lobules surrounding breast carcinomas share a number of molecular abnormalities with adjacent cancer cells such as *PTEN* deletion or *p53* mutation.[54] The detection of expanded stem-cell clusters using markers such as ALDH1 in breast biopsy tissues may identify women with increased risk of subsequent breast cancer development.[37]

Expression of Stem-Cell Markers and Prognosis

A number of studies have demonstrated that expression of stem-cell markers in mammary tumors has prognostic significance. For instance, in a series of 477 breast carcinoma patients, expression of ALDH1 was associated with poor clinical outcome.[17] Expression of this marker also identified a subset of patients with inflammatory breast carcinoma with an increased risk of recurrence. In addition, a 186-gene signature identified from $CD44^{+}/CD24^{low}$ tumorigenic cells isolated from human tumors correlated significantly both with overall and metastasis-free survival in patients with breast cancer, as well as other malignancies.[55]

Implications of the Cancer Stem-Cell Hypothesis for Breast Cancer Prevention

The cancer stem-cell hypothesis suggests that strategies targeting breast stem-cell populations may prove effective for cancer prevention and therapy. The effectiveness of prevention strategies aimed at modulating stem-cell number during key developmental windows including in utero and adolescence have been suggested by animal models. Hilakivi-Clark et al[56] demonstrated that phytoestrogens administered to pregnant mice decreased breast cancer development in their offspring. Similarly, phytoestrogens have protective effects when administered during adolescent, but not adult, stages of rodent development.[56] These studies have implications for dietary modifications in women. In addition, chemopreventive agents such as curcumin from turmeric may function by modulating stem-cell self-renewal pathways including Wnt and Notch.[57,58] In addition, several dietary polyphenoids including apple-derived quercetin, and epigallocetechin-galleate have been shown to regulate molecules in the Wnt-β catenin and Notch pathways.[59] Vitamin D_3 has also been shown to be involved in stem-cell differentiation, and therefore may have applications for cancer prevention strategies aimed at the stem cell.[60]

Metastasis and the Stem-Cell Phenotype

The most important prognostic factor influencing the outcome of patients with invasive breast cancer is whether the tumor has spread regionally or systemically.[61] There is increasing evidence that cancer stem cells play an important role in mediating tumor metastasis. As

described previously, breast cancer stem cells have been characterized as having the cell-surface phenotype $CD44^+/CD24^-$. CD44 is a cell-adhesion molecule involved in binding of cells to hyaluronic acid, whereas CD24 is a negative regulator of the chemokine receptor CXCR4, a molecule involved in breast cancer metastasis.[62] To determine the relationship of the stem-cell phenotype to metastasis, Balic et al[63] examined the expression of stem-cell markers in metastatic bone marrow sites in patients with breast carcinoma and found an increased in $CD44^+$-/$CD24^-$-expressing cells. Although the presence of micrometastasis in the bone marrow is associated with poor prognosis, approximately 50% of patients with such micrometastasis do not develop clinically apparent macrometastasis with a 10-year follow-up. Studies are currently in progress to determine whether expression of stem-cell markers in bone marrow and lymph node micrometastasis predict relapse.

We have recently demonstrated the invasive and metastatic characteristics of cancer stem cells. Aldefluor-positive populations of mammary carcinoma cell lines display increased invasive characteristics as well as increased ability to metastasize when injected into the left ventricle of NOD/SCID mice.[64]

There is increasing evidence that the tumor microenvironment plays an important role in tumor growth and metastasis. Indeed, breast density is an important risk factor for breast cancer development.[65,66] Breast density appears to be related to characteristics of breast stromal fibroblasts.[67] Growth factors produced by these stromal elements may play a role in regulating mammary stem-cell behavior. In addition to mammary fibroblasts, the role of endothelial cells and adipocytes in mammary stem cell behavior is currently being investigated.

Cancer Treatment

The cancer stem-cell hypothesis has important implications for the development of cancer therapeutics. Recent evidence indicates that breast cancer stem cells,[68] as well as cancer stem cells from other tumor types, are relatively resistant to both radiation and chemotherapy.[69] There are several postulated mechanisms for this resistance. Stem cell are slowly proliferating largely in the G0 phase of the cell cycle for extended periods of time, making them resistant to cell-cycle active chemotherapeutic agents. In addition, these cancer stem cells express increased adenosine triphosphate–binding cassette proteins known to efflux chemotherapeutic drugs. Indeed, ABCG2, or breast cancer–resistance protein, was initially identified in breast cancers. This molecule is overexpressed in stem cells and has been utilized to purify breast and other stem cells by exclusion of Hoechst dye, generating the so-called side population detected by flow cytometry.[70] In addition, enzymes such as ALDH that are highly expressed in stem cells are able to metabolize chemotherapeutic agents such as cyclophosphamide.[71] Cancer stem cells may also express increased levels of antiapoptotic molecules such as survivin and BCL2-family proteins.[72]

Current clinical trial designs have largely been based on strategies aimed at producing tumor regression. Indeed, the Response Evaluation Criteria in Solid Tumors (RECIST) criteria measuring tumor response have been used to assess efficacy of new therapeutic agents. However, in breast cancer, as is the case with other malignancies, tumor regression does not correlate well with patient survival.[61] In the neoadjuvant setting, only a complete pathologic response correlates with recurrence and survival, whereas partial response does not.[73] Together with studies demonstrating resistance of breast cancer stem cells to chemotherapy and radiation therapy, these studies suggest that limitations of present therapies may relate to their inability to target the cancer stem cell component. Recent neoadjuvant studies demonstrating an increase in the proportion of $CD44^+/CD24^-$ breast cancer stem cells after chemotherapy suggest that this is the case.[74,75] Most importantly, recent reports by Chang et al[75] suggest that targeting HER-2 with lapatinib in a neoadjuvant clinical trial was able to reduce the cancer stem-cell population, and that this resulted in a significantly increased pathologic complete response

rate. This provides strong clinical support for the cancer stem-cell hypothesis because HER-2 drives the cancer stem-cell population. The effectiveness of HER-2 inhibitors such as trastuzumab and lapatinib may relate directly to the ability of these agents to target the cancer stem-cell population. The elucidation of pathways that regulate breast cancer stem cells, such as Notch, Hedgehog, and Wnt, provide new targets for therapeutic development. Furthermore, the ability to directly measure the effect of these interventions on breast cancer stem-cell populations utilizing a neoadjuvant trial design should permit a direct test of the cancer stem-cell hypothesis. The ultimate test of this hypothesis, however, will be the demonstration that the successful targeting of cancer stem cells results in improved clinical outcomes for patients with breast cancer.

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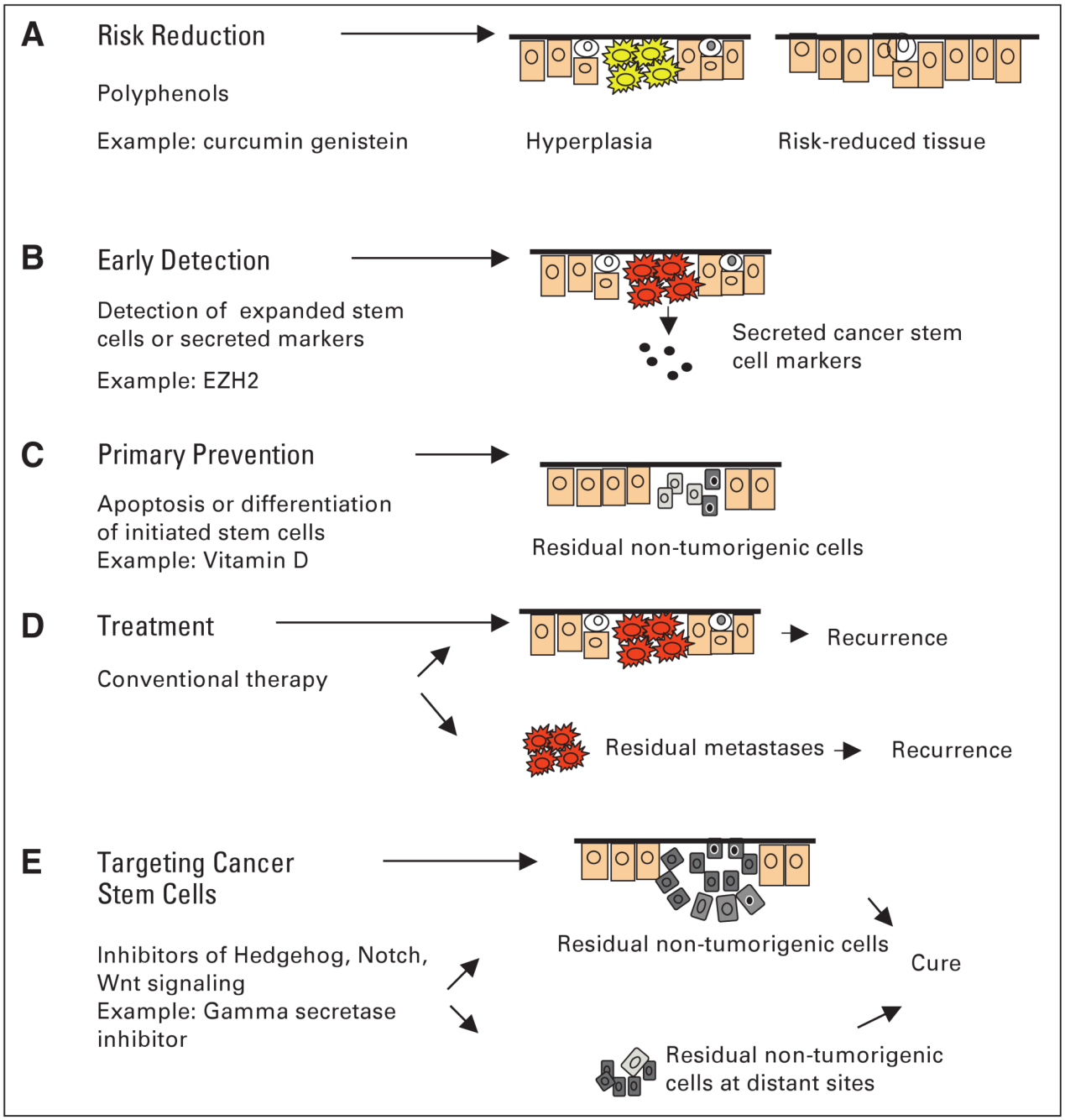

Fig 1.

Clinical interventions targeting stem cells (SCs) for cancer prevention and treatment. Cancers arise through dysregulation of SC self-renewal pathways. This produces tumors driven by a cancer SC component. Shown are strategies for cancer risk reduction, early detection, primary prevention, and treatment based on targeting the SC population. ER, estrogen receptor.

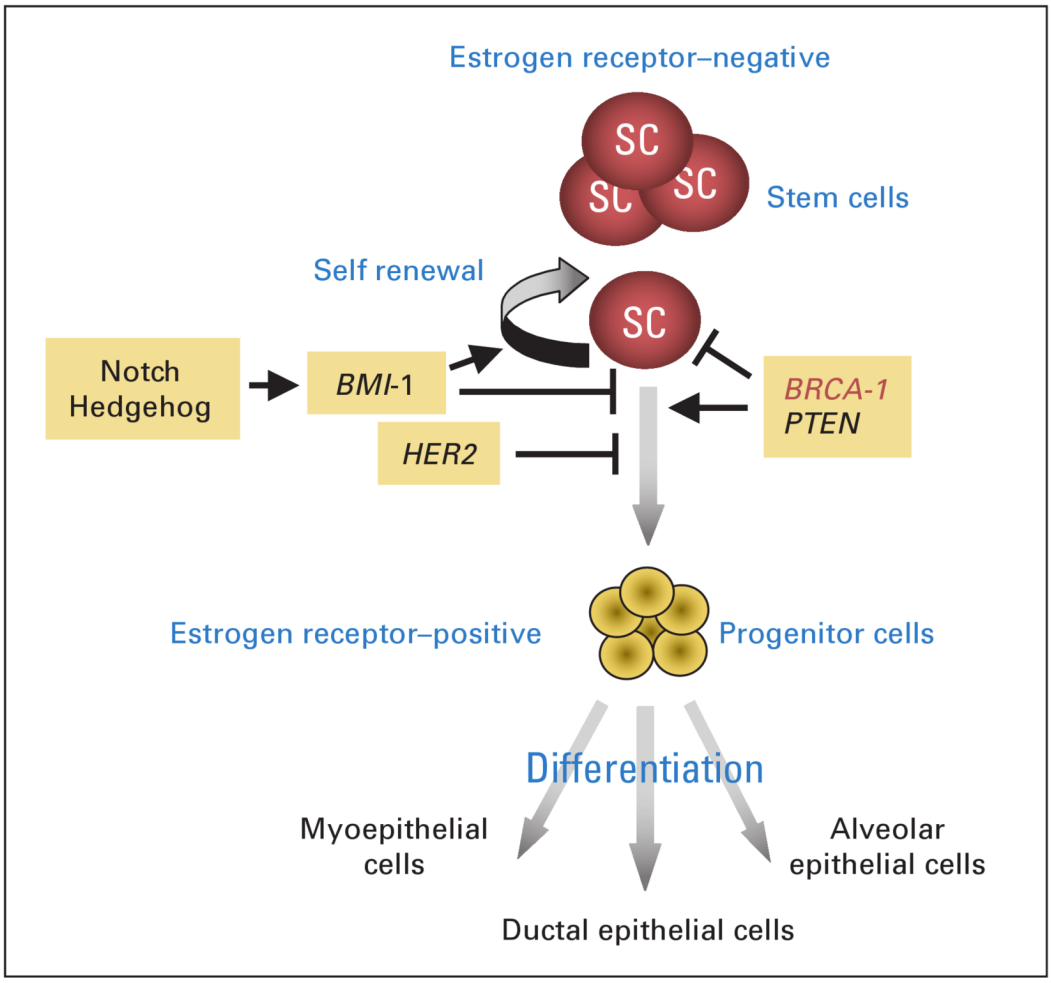

Fig 2.

Self-renewal and differentiation pathways in breast stem cells. Both hereditary and sporadic breast cancers may originate in breast stem/progenitor cells through dysregulation of the normally tightly regulated process of stem-cell self-renewal. This may result from loss of *BRCA1* function in hereditary breast cancers. In sporadic cancers this may result from loss of *PTEN* or activation of the human epidermal growth factor receptor 2, Notch, or Hedgehog pathways. This results in clonal expansion of stem cells providing targets for further carcinogenic events.