REVIEW ARTICLE

Breast Cancer Stem Cells, Pathways and Therapeutic Perspectives 2011

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Abstract The evidence for the existence of a heterogeneous population of cancer stem cells (CSCs) responsible for the initiation and maintenance of cancer has been characterized for several tumors recently. Purification and molecular characterization of normal human mammary stem cells from cultured mammospheres has been achieved, providing evidence supporting a model in which breast tumor heterogeneity is a reflection of a number of CSC-like cells in the tumor. A number of experimental methodologies have been developed to characterize epithelial stem cells, including the expression of cell surface or intracellular markers, mammosphere formation, exclusion of fluorescent dye by a side population, retention of the radionucleotide label, etc. Methodologies have also been successfully employed to identify tumorigenic cells within breast cancers. The most important characteristics of stem cells are the capacity for self-renewal and the regulation of the balance between self-renewal and differentiation. In the mammary gland, signaling pathways, such as Hedgehog (Hh), Wnt/β-catenin, and Notch, play a role in embryogenesis and organogenesis and maintenance of tissues in the adult through regulation of the balance between self-renewal and differentiation of stem cells. Breast TAAs include epitopes from proteins, such as carcinoembryonic antigen and NYBR-1, which are involved in tissue differentiation. Targeting BCSCs may be achieved by a number of approaches such as chemotherapy sensitization of BCSCs, differentiating therapy, targeting stem cell elimination, targeting signaling pathways and drug transporters, and inhibition of regulatory pathways involved in self-renewal. Targeting cells which have the potential to metastasize will be an important aspect of the BCSC field as these are the cells that cause the majority of morbidity and mortality from breast cancer.

Introduction

The classical model for cancer development holds that a series of mutations occurring in a cell can lead to cell transformation [1]. The cancer stem cell (CSC) hypothesis is based on the identification of a unique population of stem cells in the bone marrow [2]. CSCs are tumorigenic multi-potential cells with dysregulated self-renewal properties in which upon division, one daughter cell retains stemness and the other becomes committed to a lineage. The CSC fraction typically constitutes 1–5 % of the tumor size [3]. They function in initiation, maintenance, growth, and metastasis of tumors [4, 5] and demonstrate slow cycling and indefinite ability to renew themselves [6]. CSCs can proliferate limitlessly and are more resistant to chemotherapy and apoptosis than somatic cancer cells [7]. The evidence for the existence of a heterogeneous population of adult stem cells responsible for the initiation and maintenance of cancer has been characterized for several tumors recently [8–11]. Pece and colleagues reported the purification and molecular characterization of normal human mammary stem cells from cultured mammospheres, and provided evidence supporting a model in which breast tumor heterogeneity is a reflection of the number of CSC-like cells in the tumor [12]. Researchers have recognized that CSCs comprise not only CD133+ population, but also a heterogeneous CD133− population

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expressing a wide range of markers [13]. It is generally agreed that CSCs are organized into a hierarchy consisting of cancer "stem cells" at the top of hierarchy capable of sphere formation, and cancer progenitor cells lower in the hierarchy that are incapable of sphere formation but still retain the ability to initiate tumors [9]. A study published by Chen et al., in 2010, presented compelling evidence that CSCs are organized in a three-tiered hierarchy similar to endogenous stem cells, where "stem cells" higher up in the hierarchy could differentiate into a more lineage restricted genitor populations at the bottom of the hierarchy [14]. They further observed that cancer stem cells higher up in the hierarchy are generally slow-dividing sphere forming cells, but gives rise to expansive infiltrative tumor growth in vivo that is well-correlated with high tumor grade. An alternative hypothesis called the "clonal evolution of tumors theory" postulates that cancer originates from mutations occurring in a few cells or a single cell that eventually leads to genomic instability, resulting into uncontrolled and unlimited proliferation of a population of cells [15].

Liang and colleagues [16] demonstrated that drug-induced genomic instability in cancer cells can drive the emergence of CD133+ CSCs. Sharma et al. [17] showed that these CSC populations are not a fixed population, but rather a volatile population arising from the profound epigenetic instability of cancer cells and that the cancer cell stemness, and concomitant resistance to chemotherapy can be turned on and off through DNA methylation, a process through which gene expression is regulated by chemical modification of the chromatin structure suggesting that the tumor stem cell phenotype is highly volatile, and can emerge from seemingly non-stem cancer cells.

Characterization of Epithelial Stem Cells

A number of experimental methodologies have been developed to characterize epithelial stem cells, including the expression of cell surface or intracellular markers, mammosphere formation, exclusion of fluorescent dye by a side population, retention of the radionucleotide label, etc. Methodologies have also been successfully employed to identify tumorigenic cells within breast cancers. Al-Hajj et al. initiated tumors in immunodeficient mice with cells derived from human breast cancer-associated malignant pleural effusions [11]. It was also observed by them that a small proportion of tumorigenic cells could be isolated from dissociated tumor cells by flow cytometry. Following removal of nonepithelial cells, cells with the CD44+CD24−/loLin- phenotype were 10 to 50-fold enriched in their ability to initiate tumors as well as transfer the tumor to secondary and subsequent hosts as compared to unsorted cells,

demonstrating the capacity for self-renewal. HER2 over-expression has been associated with increased expression of the stem cell marker aldehyde dehydrogenase and an increase in fractions of stem cell progenitors in breast tumors [5]. Self-renewal has also been demonstrated by dissociation of mammospheres, reculture, and then reaggregation, and cells from the secondary mammospheres and beyond were almost exclusive bipotent or tripotent [10]. Mammospheres initiated tumors in the cleared mammary fat pad of immunodeficient mice at 1000-fold greater dilutions than established breast cancer derived cell lines, and the breast cancer cell with the capability for long-term self-renewal, enriched within the CD44+CD24− subset, was also seen [10, 11].

Tumors induced by expression of Wnt pathway express markers of both luminal and basal/myoepithelial lineages [18] and suggest origin from a pluripotent precursor, presumably a mammary epithelial stem cell [19]. This is not seen with other oncogenes such as Neu and H-Ras [20]. Members of the transforming growth factor-β (TGF-β) superfamily, which include bone morphogenetic proteins (BMP), are involved in the control of many different biological processes, including cell proliferation, differentiation, apoptosis, and regulation of invasiveness [21]. TGF-β can also potentiate tumorigenesis and contribute to the progression and invasiveness of various carcinomas [22]. Blockade of TGF-β (signaling) inhibits tumor cell viability, migration, and metastasis [23], including the formation of bone metastases [24]. BMP-7 is expressed in various cell lines, but in a cell line specific manner as the breast cancer cell lines MCF-7 and SK-BR-3 showed

BMP-7 expression on the mRNA level [25]. BMP7 has been proposed as a marker of differentiation in normal and breast cancer cells [26]. BMP-6 and BMP-7 inhibit estrogen-induced proliferation of breast cancer cells by suppressing p38 MAPK activation [27].

Mapping the transcriptional profile of embryonic stem cell-like genes in primary human breast cancer has revealed two classes of tumors: those with an embryonic stem cell-like activated program and those with an embryonic stem cell-like repressed program [28]. Those tumors with an embryonic stem cell-like activated program were associated with poorer differentiated tumors that were more likely to progress to metastasis and death. The CD44+/CD24 low phenotype in human breast tumors has been found to be associated with basal-like tumors, and particularly BRCA1 hereditary breast cancer, and has been linked to expression of CD49f, elevated expression of CK5/14 and EGFR, and low expression of ER, PgR, and HER-2 [29]. Basal-like tumors often have been linked to poorer prognosis. The occurrence of the CD44+/CD24 low phenotype was found to be lower in tumors of luminal type, and particularly HER-2+ tumors, irrespective of ER status [30].

The Epithelial–Mesenchymal Transition Phenomenon

Epithelial–mesenchymal transition (EMT) consists of a rapid and often reversible change of cell phenotype. Epithelial cells loosen cell–cell adhesion structures including adherens junctions and desmosomes, modulate their polarity, and rearrange their cytoskeleton; intermediate filaments typically switch from cytokeratins to vimentin. Cells become isolated, motile, and resistant to apoptosis. In vivo, the EMT process generates poorly differentiated and potentially pluripotent cells rather than true fibroblasts, a differentiated cell type expressing tissue and organ specificity. This intermediate phenotype is linked to the expression of basal cytokeratins (CK5, CK14) and to some level of cell–cell interaction allowing group cell migration [31]. The EMT was initially defined as the cellular remodeling occurring during heart morphogenesis, but the concept was extended to analogous transformation occurring during the formation of mesoderm and neural crest [32, 33], wound healing, mammary tubulogenesis [34], and stemness [35, 36]. Many genes and pathways have been implicated in inducing EMT in tumor cells involving so-called EMT master genes, genes of a family of transcriptional factors including Snail, Twist, Zeb, and E47 [37]. In direct association with cancer progression, several molecules including tyrosine phosphatase Pez [38], ILEI [39], RKIP [40], and CXCR447 appear to control EMT-like phenotypes and tumor metastasis in mouse models [37].

Both human tumors and mouse models of breast tumorigenesis show evidence of EMT or partial EMT. Besides the modification of the phenotype, EMT results in the acquisition of other properties involved in carcinoma progression, such as an increased ability to migrate, a higher resistance to apoptosis, and the already mentioned acquisition of stemness properties [31]. Invasive breast carcinomas are characterized by their strong heterogeneity, reflecting tumor histology and response to therapy. It is likely that the contribution of a process like EMT in cancer progression depends on the tumor type [31]. The best case for a complete EMT taking place during mammary tumor progression is carcinosarcoma or metaplastic carcinomas, where an epithelial and a mesenchymal compartment can be distinguished based on the expression of, respectively, cytokeratins or vimentin intermediate filaments [31]. Cytogenetic studies strongly indicate that these two compartments originate from a common precursor cell population undergoing a full EMT process, giving rise to the mesenchymal component [41]. Recent studies show overexpression of Snail genes in these tumors, correlating with activation of Akt and β-catenin pathways [42]. A more prevalent mammary tumor, the infiltrating lobular carcinoma, is also characterized by the lack of E-cadherin expression, reflecting genomic and epigenetic silencing mechanisms [43, 44]. These tumors express significantly higher levels of a "classic" EMT master gene, Twist [45].

Role of microRNAs in Breast Cancer

microRNAs are noncoding RNA molecules that exert their effect at the translational level and are valuable in regulating gene expression [46]. The Tac1 gene, which is linked to breast and other cancers, could be suppressed by translational inhibition [47]. Three miRNAs have been found that may bind to Tac1: miR-130a, miR-206, and miR-302a [48]. Tac1 regulates breast cancer cell interaction with the mesenchymal stem cells, and microRNAs against Tac1 may affect quiescence of breast cancer cells in the marrow cavity [49].

Breast Cancer Metastatic Pathways

Metastatic spread of tumor cells can be an early event in tumorigenesis [50]. It has been experimentally demonstrated that a small tumorigenic cell population exists in solid tumors of both the breast and the brain which represent around 1–2 % of the total tumor burden and these cells might have a phenotype different from the original tumor-initiating cells [11]. Circulating tumor cells can often be detected in patients with both primary and metastatic diseases, and the presence of these cells is often associated with relatively worse prognosis for survival [51]. CXCR4, a chemokine receptor, expressed by hematopoietic stem cells which bind CXCL12, has been shown to be increased by about four times in mammospheres to be expressed in both metastatic breast cancer cells and neuroblastomas [52]. Additionally, the organs that form the main target of breast cancer metastasis have the highest expression of the ligand CXCL12 [52]. Recently, researchers have identified a subset of CSCs, present in all terminal colon cancer cells and all metastatic cancer cells, having metastatic capacity with a surface marker CD26 [53].

Steroid Hormone Receptor Expression of the Breast Cancer Stem Cells

Recently, it has been shown that the mammary reconstituting cells are ER − and PgR − [54]. Recent experiments have demonstrated a role for BRCA1 being involved in the differentiation of human ER − stem/progenitor cells into ER + luminal epithelial cells [55]. The deletion of BRCA1 results in the prevention of the transition of ER − stem cells into ER + progenitor cells, and heterozygous mutations in the BRCA1 gene predispose women to breast and ovarian cancer, with tumors often being of the basal-like phenotype characterized by a lack of expression of ER, PgR, and HER-2 [56]. A model of breast cancer origin has also been proposed in which ER + tumors are derived from ER + stem cells or ER + early or late progenitor cells and ER − tumors are derived from the more primitive ER − stem cells [57].

Other models have postulated that ER − stem cells, which rarely divide and are also resistant to hormonal therapy, can generate ER + short-term transit-amplifying cells that in turn give rise to ER + differentiated cells [30].

Self-renewal and Differentiation Pathways of Stem Cells

The most important characteristics of stem cells are the capacity for self-renewal and the regulation of the balance between self-renewal and differentiation [30]. In the mammary gland, signaling pathways, such as Hedgehog (Hh), Wnt/β-catenin, and Notch, play a role in embryogenesis and organogenesis and maintenance of tissues in the adult through regulation of the balance between self-renewal and differentiation of stem cells. The Notch family of transmembrane signaling proteins is involved in cell fate development and expressed in stem cells and early progenitor cells [58]. Notch-4 has been shown to suppress differentiation of breast epithelial cells in vitro [59]. A Notch-activating peptide increased secondary and tertiary mammosphere formation tenfold and increased the proportion of cells with multilineage differentiation capacity hundredfold, implying increased mammary stem cell self-renewal and also suggesting that alterations in Notch-4 signaling might play a role in the transition of a healthy stem cell to a CSC [60]. β-Catenin, a downstream target of Wnt signaling pathway, has been identified as a crucial survival signal for mammary epithelial stem cells [61]. Overexpression of Wnt in mouse mammary glands can also lead to increased mammary tumor formation [62]. Mammary tumors arising in mice overexpressing components of this pathway contain cells of both basal/myoepithelial and luminal phenotypes, suggesting an origin from a common precursor [18]. The PITCH membrane protein is a receptor for the Hedgehog (Hh/Patched pathway) family of signaling molecules and has been connected to early embryonic tumorigenesis [63]. Overexpression of components of this pathway, including Shh, Ptch1, and Gli1, is found in a large majority of human breast cancer specimens but not in adjacent normal epithelium consistent with constitutive activation of this pathway [64]. In mice, Ptc-1 haplo-insufficiency leads to abnormal mammary development with ductal dysplasia and hyperplasia by 5 weeks of age [65]. Gli1 heterozygosity results in abnormal mammary development with ductal dysplasia in parous animals [66].

Breast Tumor-Associated Antigens

Breast tumor-associated antigens (TAAs) include epitopes from proteins, such as carcinoembryonic antigen and NYBR-1, which are involved in tissue differentiation [67].

They are overexpressed in breast cancer, or they are shared among a variety of tumors [68]. Breast carcinomas fall into various subtypes associated with different gene expression patterns, phenotypes, and clinical outcomes [69]. However, in a single patient, tumors are heterogenous, with individual tumor cells displaying different phenotypes and TAAs [30].

Mammospheres

Mammospheres have shown expression of markers ESA, CK5, and α6-integrin among many others, which potentially could be used to identify or target breast cancer stem cells (BCSCs) [45, 60]. The activity of aldehyde dehydrogenase-1 (ALDH1), a detoxifying enzyme that may play a role in the differentiation of stem cells, has been detected in both normal and malignant human mammary stem cells and can be used as a predictor for poor clinical outcomes [70]. CD44, another molecule used to identify or target BCSCs, is a membrane receptor involved in cell adhesion, motility, and metastases [71]. CD44 has routinely been used as a marker to purify and enrich BCSCs by selecting for cells that are CD44+CD24−/lowLin- and ESA + [11]. CD29 (β1-integrin) and CD49f (α6-integrin) expression has also been associated with murine mammary stem cells with a Lin-CD24+ phenotype [20]. Additionally, neither Sca-1 (stem cell antigen) expression nor the side population (SP) phenotype was found to be expressed in the mammary reconstituting Lin-CD29hiCD24+ cell population [20]. However, one report using the human breast cancer line MCF7 has shown that the SP phenotype can be used to identify cells with characteristics of CSCs that express the tumor antigen MUC1, supporting a role for the SP in further analysis of human BCSCs [72]. A recent report has shown that BRCA1-deficient murine breast tumors contain heterogeneous CSC populations [73].

Therapeutic Perspectives Targeting BCSCs

As compared to most cancer cells, CSCs are slow-dividing, have a lower ability to undergo apoptosis and a higher ability of DNA repair, making them more resistant to traditional methods of cancer treatment such as radiation and chemotherapy [30]. In vitro experiments comparing differentiated breast cancer cells grown under monolayer conditions with CD24−/low CD44+ CSCs grown under mammosphere conditions showed that the stem cell-like population was more resistant to radiation [74]. In addition, stem cells express ABC drug transporters, which protect the cell from cytotoxic agents and may lead to multidrug resistance [75]. Targeting BCSCs may be achieved by a number of approaches.

Chemotherapy Sensitization of BCSCs

A wide variety of pathways have been implicated to sensitize BCSCs to the existing chemotherapy, including decreased topoisomerase II expression, reduced sensitivity to apoptosis via alterations in the expression of Bcl2 family members, efflux of chemotherapeutic agents from cancer cells for multidrug resistance, etc. [76]. Several of these mechanisms, in particular resistance to DNA damage and the efflux of toxic compounds, are also advantageous in tissue stem cells, and their activation in malignancy may reflect not only somatic mutation but also their intrinsic activation in the tissue stem cells from which cancer arises. Moreover, as CSCs are at the top of the differentiation hierarchy, they cycle less frequently than other tumor cells and therefore have a greater resistance to antimitotics [19]. Upregulation of cell surface transporters of the ABC family, in particular ABCG2 (breast cancer resistance protein, BCRP), was initially identified as a transcript overexpressed in multiresistant breast cancer [77]. Promising inhibitors for another ABC family transporter implicated in multidrug resistance, P-glycoprotein, are also entering clinical trials [78].

Differentiating Therapy

After debulking of differentiated tumors, targeting of the remaining surviving, often quiescent, CSCs can be achieved by differentiating BCSCs through differentiating therapy or eliminating them via immunotherapy. Retinoic acid and other vitamin A analogues can promote differentiation of epithelial cells and reverse tumor progression through modulation of signal transduction [79]. During metastasis, migrating BCSCs undergo a loss of polarity leading to an epithelial to mesenchymal transition (EMT). Gupta et al. used this attribute of CSCs to develop a high-throughput screen, which successfully identifies small molecules that specifically inhibit CSC proliferation through the induction of differentiation [80]. BMP7 is a potent inhibitor of TGF-β-induced EMT in MDA-231 cancer cells. Decreased BMP7 expression during breast cancer progression may contribute to the acquisition of a bone metastatic phenotype [25].

Targeting Stem Cell Elimination

Present cancer therapy research is mostly focused on targeting specific markers on tumor cells that are overexpressed or mutated and that often represent essential genes/proteins or pathways thought to be important for the development of the tumor.

Traztuzamab targets the HER-2/neu (ErbB2) oncogene which is a member of the epidermal growth factor receptor (EGFR) kinase family and a protein overexpressed on nearly 30 % of breast tumors [81]. Tumors may be not only driven by mutated proteins and inappropriate signaling, but also that epigenetic mechanisms of gene expression of genes involved in "stemness" such as Oct4, Nanog, and Sox2 could be behind tumor formation [82]. Reversal of these epigenetic switches of CSCs could be one novel way to target CSCs [30].

Targeting Signaling Pathways and Drug Transporters

In the mammary gland, Notch, Hedgehog, and Wnt/β-catenin are the three signaling pathways which play a role in stem cell self-renewal and thus represent potential targets for therapy for BCSCs, along with targeting the ABC drug transporters that cancer stem cells use to evade chemotherapy. The use of the cyclopamine to inhibit the Hh signaling pathway has shown some promise in inhibiting the growth of medulloblastoma and could be used in treatments of other tumors [79]. Gunther and colleagues utilized a doxycycline-inducible wnt-1 transgene to show that withdrawal of wnt signaling is sufficient to induce regression of primary mammary tumors and pulmonary metastases [83]. The Wnt pathway can also be inhibited through a variety of other mechanisms. Targeting of β-catenin has received a lot of attention as RA has been shown to inhibit β-catenin activity [84], and tyrosine kinase inhibitors such as imatinib have been shown to downregulate β-catenin signaling [85]. The effects of Notch signaling on stem cells to promote self-renewal and on progenitors promoting their proliferation were completely abrogated by the use of a Notch-4 blocking antibody [86].

Inhibition of Regulatory Pathways Involved in Self-renewal

Recent clinical evidence has established that tumorigenic breast cancer cells with high expression of CD44 and low expression of CD24 are resistant to chemotherapy [87]. Breast cancer patients receiving neoadjuvant chemotherapy had an increase in the CD44+/CD24 low population of cells following treatment. These cells retained the capacity to form mammospheres, thus demonstrating self-renewal, and had an enhanced propensity for forming tumors in SCID/Beige mice compared with pretreatment samples [19]. Treatment of patients with HER-2-positive tumors with lapatinib, an EGFR and HER-2/neu (ErbB-2) dual-tyrosine kinase inhibitor, resulted in nonstatistically significant decreases in the percentage of CD44+/CD24 low population and in the ability for self-renewal as assessed by mammosphere formation [19]. It has been observed that blockading interleukin 8 (IL-8) receptors, such as CXCR1 with the

CXCR1 inhibitor repertaxin and alternatively a CXCR1-specific blocking antibody, inhibit some human BCSCs [88].

Immunotherapy Targeting BCSCs

Molecular identification of TAAs can be used as targets for therapy. Dendritic cells (DCs) are professional antigen-presenting cells and initiators of adaptive immunity through processing antigens and presenting epitopes in the context of major histocompatibility complex (MHC) to T cells and play central role to these processes as a result of their role in innate immunity and in generating humoral and cellular immune responses [89]. Evidence for the protective role of the immune system against cancer is seen in the increased incidence of melanoma observed in renal transplant recipients [90]. For breast cancer, it has been observed that DCs and lymphocytic infiltrates in tumors are associated with better prognosis and survival, independent of tumor size [91]. However, in breast cancer, it is also apparent that DC impairment correlates with tumor progression. For instance, co-stimulatory molecule expression and antigen presentation by DCs are decreased in breast cancer patients, with a subsequent impairment in the capacity of these DCs to stimulate T-cell proliferation and secretion of cytokines [92]. DCs of breast cancer patients secrete less interleukin-12 in response to maturation signals [93]. Interestingly, it has also been shown that breast cancer can induce apoptosis in circulating DCs but this process is reversible when the DCs are removed from the inhibitory cancerous environment [94]. These findings suggest a strong role for the immune system in both the progression of disease and as a potential tool to eliminate cancer. Dendritic cell (DC)-based vaccination strategies encompass a variety of different approaches that can be grouped into antigen-defined vaccines and polyvalent vaccines [95]. In one preclinical mouse model, a dendritic cell-based vaccine prevented the outgrowth of a spontaneous breast tumor when used to specifically target a single differentiated HER-2/neu tumor antigen [96]. This vaccine induced the production of anti-neu antibodies and interferon-γ expression by T cells. Although, DC-based vaccines have no serious side effects [97], overall clinical trials with DC-induced antitumor T-cell responses have been disappointing [80, 95]. Most clinical trials using DCs have examined the use of single antigen peptide-loaded DCs [98, 99]. In one trial, using tumor necrosis factor-alpha-matured, monocyte-derived DCs pulsed with MUC1, or HER-2/neu elicited peptide-specific anti-tumor responses, the overall clinical response was modest [100]. The adoptive transfer method has already been used with some success to target cancer [101]. Gene expression comparisons have been conducted and can be used to identify what genes are expressed by the CSC compartment compared with normal stem cells [102].

Chemotherapy Targeting CSCs

Multiple characteristics of CSCs account for resistance to chemotherapy and radiation, including lack of a targetable phenotype and specific oncoprotein expression, high level of MDR1 expression, slow cycling rate, etc. [3]. The use of monoclonal antibodies to target CSCs has been proposed based on the ideas that antibodies can interfere with cancer

Fig. 1 Diagrammatic presentation of normal mammary stem cell and breast cancer stem cell
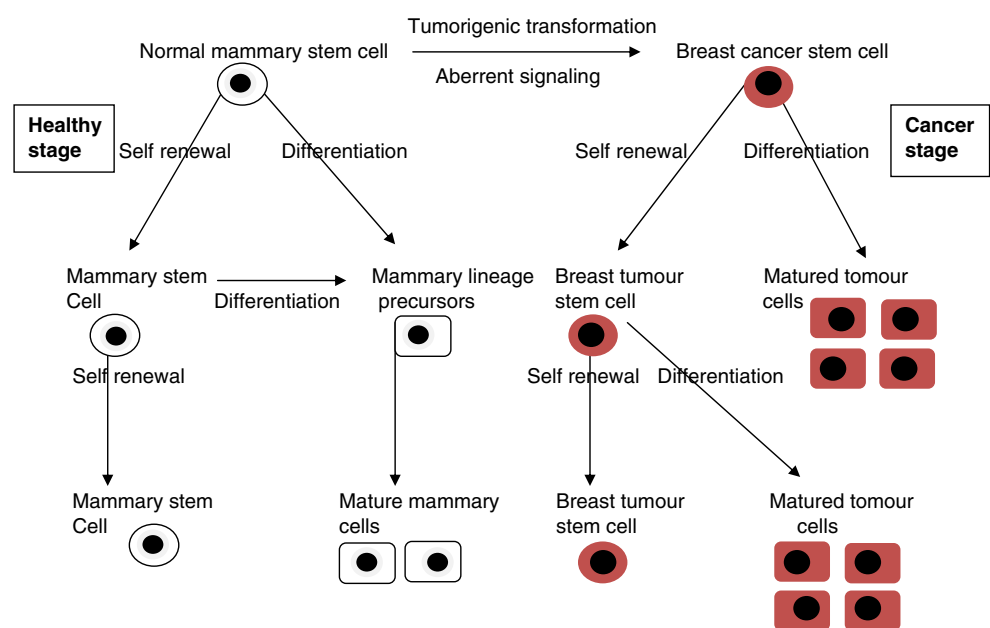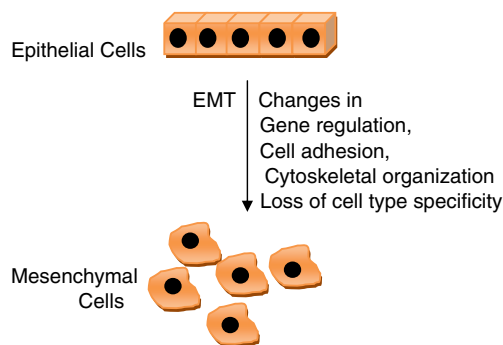
Fig. 2 Diagrammatic presentation of EMT phenomenon

cell signaling pathways, assist in the delivery of anticancer agents, and facilitate an immune response to tumors [5]. Recently, a compound derived from broccoli, sulforaphane, could help prevent or treat breast cancer by targeting CSCs. Researchers found that sulforaphane targeted and killed CSCs and prevented new tumors from growing [103]. Decitabine, a DNA hypomethylating agent, may target not only active ovarian cancer cells but also CSCs that seem to survive the first treatments [104] (Figs. 1 and 2; Tables 1 and 2).

Targeting Metastatic Disease

Targeting cells which have the potential to metastasize among BCSCs will be an important aspect as these are the cells that cause the majority of morbidity and mortality from breast cancer. Chemotherapy shows limited success to target quiescent breast cancer cells in the bone marrow, and several mechanisms of dormancy, as well as tertiary metastasis from the bone marrow, have been proposed [105]. The NK1 receptor is constitutively expressed in neural tissue but

Table 1 Breast cancer stem cell (BCSCs) markers

BCSCs marker	References
CD44+CD24-/loLin- phenotype	[11, 29, 30]
HER2 overexpression	[5]
Mammosphere formation	[10, 11]
Exclusion of fluorescent dye by a side population	[113]
Transforming growth factor-β (TGF-β)	[22]
BMP-7 expression	[25–27]
microRNAs	[33, 34]
CXCR4 (Chemokine receptor)	[37]
CXCL12 expression	[37]
ESA, CK5, and α6- integrin	[45]
Aldehyde dehydrogenase-1 (ALDH1) activity	[55]
CD44	[11]

Table 2 Therapeutic approaches targeting breast cancer stem cells

Approach	Targeting pathways/agents
Chemotherapy sensitization of BCSCs	Decreased topoisomerase II expression
	Reduced sensitivity to apoptosis
	Efflux of chemical agents
	ABCG2
	P-glycoprotein
Differentiating therapy	Vitamin A analogues
	BMP7
Targeting stem cell elimination	Traztuzamab
	Epigenetic mechanisms of gene expression
Targeting signaling pathways and drug transporters	Notch, Hedgehog, and Wnt/β-catenin
	ABC drug transporters
Inhibition of regulatory pathways involved in self-renewal	Lapatinib
	Repertaxin
Immunotherapy targeting breast cancer stem cells	Dendritic cell (DC)-based vaccination
Chemotherapy targeting cancer stem cells	Monoclonal antibodies
	Sulforaphane
	Decitabine
Targeting metastatic disease	NK1 receptor
	Tac1 gene
	CXCR4 antagonists

inducibly expressed in bone marrow cells and breast epithelial cells [106]. Interactions between peptides of Tac1 gene and NK1 are involved in hematopoietic regulation, depending on the interacting peptide or the signaling receptor [106]. In nontumorigenic MCF12A breast cells, activated nuclear factor-κB (NF-κB) has been shown to suppress Tac1 expression level in the presence of high levels of SDF-1α [49]. Thus, the NK1 receptor offers a valuable pharmacologic target in diseases such as breast cancer, neuroblastoma, and hematological malignancies [107]. Recent investigations on the mechanisms of breast cancer cell metastasis to the marrow cavity have implicated a central role for Tac1. Tac1 appears to regulate the expressions of SDF-1α and CXCR4 on both cancer cells and mesenchymal stem cells [49]. These findings could lead to future studies to define a new method of treatment by targeting the SDF-1α-CXCR4 interactions, and also to target the Tac1 gene. Such treatments could be possible in the near future due to the availability of CXCR4 antagonists [49]. Genes linking to various stages of breast cancer in bone marrow has also been proposed [108]. The promotion of apoptosis by TGF-β has been linked to its interactions with the Survivin gene. TGF-β can downregulate

the expression of Survivin at the level of gene transcription, resulting in apoptosis [109]. TGF-β also has the ability to arrest the cell cycle progression in G1 phase via the pRb tumor suppression mechanism, thereby preventing the S phase entry and breast cancer cell proliferation [110]. However, under the regulation of c-myc, breast cancer cells become less susceptible to the effects of TGF-β [110]. Both NK1 and hematopoietic growth factor inducible neurokinin-1 type (HGFIN) have been linked to tumorigenesis, including breast cancer [107]. HGFIN is a type I transmembrane glycoprotein that maps to the short arm of chromosome 7 and shares structural homology to the NK1 receptor and murine osteoactivin [107]. However, their roles are contrasting. While NK1, in its truncated form, exerts oncogenic properties, HGFIN shows tumor suppressor roles [107]. In breast cancer cells, HGFIN suppresses their growth and migration [48].

Conclusion

There is substantial evidence that proliferation within the normal mammary epithelium is organized in a hierarchical manner. Repopulation experiments and lineage analysis support the existence of pluripotent mammary epithelial stem cells, and cell surface markers have been identified to facilitate their purification. Understanding of the biology of BCSCs should better enable the design of therapies targeted at these CSCs. Gene profiling is a powerful tool in identifying different types of breast cancer with respect to response to therapy, relapse, and metastatic potential. Additionally, targeting universal TAAs such as human telomerase reverse transcriptase and inhibitor of apoptosis proteins might be important for effectively targeting tumors with immunotherapy, as will combining these treatments with ones that target unique stemness-related antigens [111]. Several additional markers and signaling molecules, such as the Hh/patched pathway, as well as the ABCG2 drug transporters could be used as potential targets of therapy for breast cancer [112]. Additionally, markers that are used for migration of BCSCs, such as chemokine receptors, should be explored as potential targets.

In the field of stem cell-targeted therapy, appropriate preclinical models to determine the efficacy of cancer treatment are lacking. It may be important in future studies designed to identify human BCSCs or initiating cells to use improved mouse xenograft models which mimic the human breast microenvironment. Once unique pathways are identified, combination therapy targeting multiple pathways may be more effective than single therapy, based on the observation that CSCs may be heterogeneous with respect to their quiescence state and proliferation capacity as well as the mechanisms underlying their transformation.

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