Review Article

Stem Cell INVESTIGATION

Breast cancer stem cells and the challenges of eradication: a review of novel therapies

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Abstract: Breast cancer is a heterogeneous disease that accounts for 30% of all cancers diagnosed in women and over half a million deaths per year. Cancer stem cells (CSCs) make up a small subpopulation of cells within a tumor, are capable of self-renewal and, are responsible for tumor initiation, formation, and recurrence. Breast CSCs (BCSCs) have been the subject of concentrated research as potential targets for breast cancer therapies. Cell surface markers CD44+/CD24– have been established as minimum biomarkers for BCSCs and the upregulation of CD44 expression has been linked to tumor formation in numerous cancers. Additionally, the deregulation of Notch, Wnt/Frizzled/β-catenin, Hippo, and Hedgehog signaling pathways is believed to be responsible for the formation of CSCs and lead to tumor formation. Tumor heterogeneity is a key feature of therapy resistance and a major challenge. CSCs are predominantly senescent and inherently immune to chemotherapy drugs which rely on an overactive cell cycle. Current therapeutic strategies include targeting CSC signaling pathways that play critical roles in self-renewal and defense. Anti-CD44 antibodies have been shown to induce terminal differentiation in CSCs resulting in a significant decrease in tumor metastasis. Additionally, targeting the tumor microenvironment has been shown to increase the effectiveness of chemotherapy drugs. In this review, we attempt to provide an overview of breast cancer, the stem of its cause, and novel therapies currently being explored.

Keywords: Cancer stem cells (CSCs); signaling pathways; novel therapeutics; breast cancer

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Prevalence of breast cancer

Breast cancer accounts for 30% of all cancers diagnosed in women with greater than 1,677,000 new cases and over 520,000 deaths per year worldwide (1). Despite recent advancements in detection and treatment, mortality of the disease is expected to increase 20% by the year 2020 with >95% of new cases occurring in women older than 40 years of age (2,3). Approximately 40% of patients who are initially diagnosed with non-invasive breast cancer progress to malignancy and experience disease recurrence despite undergoing treatments such as chemotherapy and/or adjuvant care. Furthermore, 70% of these cases experience a metastatic relapse within 5 years (4). Due to the heterogeneous nature of this disease, the effectiveness of recent therapies has been limited (5).

In this review, we provide a clinical discussion of metastatic breast cancer including a review of the breast cancer stem cell (CSC), its signaling pathways and immunological/pathological markers, and novel therapies designed for targeted treatment.

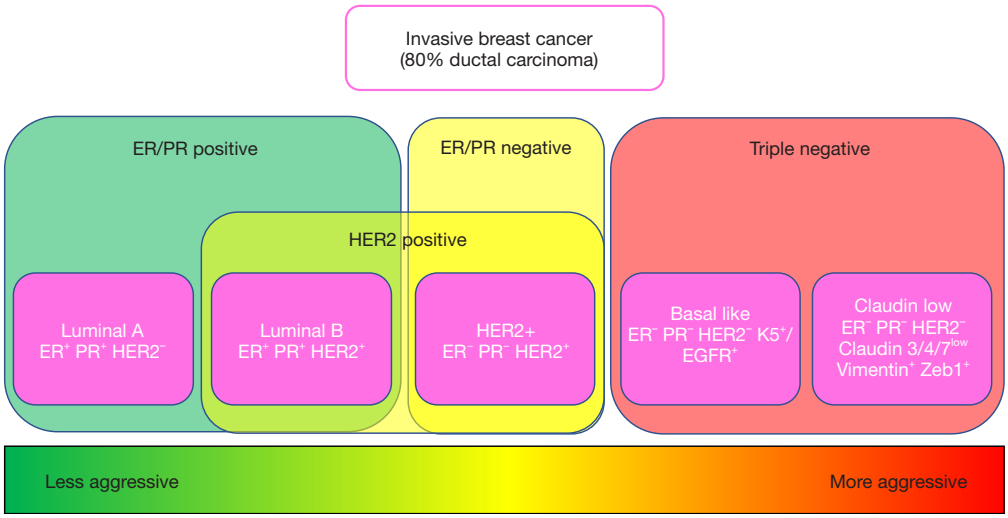

Figure 1 Comparison of the current breast cancer subtypes according to expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal receptor 2 (HER2). Based on positive expression of ER, PR and/or HER2 breast cancer subtypes are classified as ER/PR positive, ER/PR negative and/or HER2 positive. Negative expression of ER, PR, and HER2 designates the subtype as "triple negative", typically associated with a more aggressive tumor and a poorer prognosis.

Types of breast cancer

Clinical character

Breast cancer is a molecularly, pathologically, and epidemiologically heterogeneous disease. Clinically, invasive breast cancers can be classified into three groups: early breast cancer (stages I, IIa, and IIb), locally advanced breast cancer (stages IIIa, IIIb, and IIIc), and advanced breast cancer (stage IV) presenting with distant metastases beyond the regional lymph nodes (6,7).

Immunological subtype

Markers in breast cancer have long been appreciated by researchers to define and identify options for targeted treatment. There are four immunological subtypes of breast cancer containing a combination of the three chief markers; estrogen receptor (ER), progesterone receptor (PR), and human epidermal receptor 2 (HER2) which are routinely defined in the clinic in order to optimize patient outcomes (8).

Gene expression profile

Global gene expression analyses have given us a closer look at this complex heterogeneous disease. Six intrinsic molecular subtypes (luminal A, luminal B, basal-like, HER2-overexpressing, Claudin-low, and normal breast-like) of breast cancer have been identified and have provided a deeper understanding of the differences in gene expression between tumors presenting with these variable immunological markers (9,10). For example, there is clear evidence that "basal-like" and "luminal" subgroups differ with respect to outcome of disease in patients with locally advanced breast cancer (11) (*Figure 1*).

CSCs

Cancers are believed to arise from a series of genetic mutations that occur as a result of cellular instability and/or oncogene-induced plasticity (12). First discovered in acute myeloid leukemia (AML), CSCs have played a major role in the advancement of cancer research (13). CSCs have led to a remodeling of our cancer hypothesis and have been the subject of concentrated research as potential targets for cancer therapies (14-18). The majority of cells within solid tumors are more differentiated and have limited self-renewal abilities (19). CSCs, on the other hand, make up a small subpopulation of cells within a tumor and are responsible for tumor, initiation, formation and recurrence (14). CSCs have been shown to undergo symmetric self-renewal giving rise to two identical pluripotent daughter CSCs, as well as, an asymmetric division producing a more differentiated tumor progenitor cell (TPC) and an identical daughter CSC. This self-promoting mechanism results in an increased number of

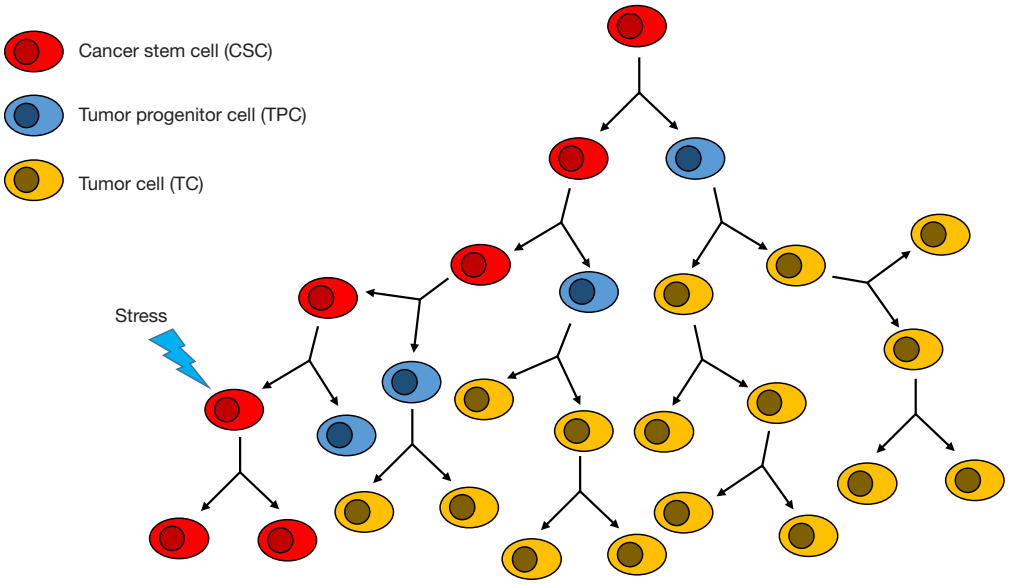

Figure 2 Cancer stem cells (CSCs) divide asymmetrically and give rise to one copy of self and one differentiated tumor progenitor cell. Tumor progenitor cells are no longer able to self-renew, they undergo symmetric division and form the terminally differentiated cells of the bulk tumor mass. Under stress, CSCs may undergo symmetric self-renewal increasing tumor resistance to therapy.

CSCs as the tumor grows as well as expansion of the overall tumor in size (20). Furthermore, CSCs have been shown to undergo epithelial-to-mesenchymal-transition (EMT), a known mechanism in metastasis (21) (*Figure 2*).

Breast CSCs (BCSCs)

BCSCs are derived from human breast tumors with a series of markers, including CD44, CD24, CD133, epithelial cell adhesion molecule (EpCAM), nestin, ganglioside GD2, CD49f, CD61, CXCR4, CXCL1, HMGCS, CD166, CD47, ALDH1, and ABCG2 (22-25). However, cell markers CD44+/CD24– have been established as minimum surface markers for BCSCs (14). CD44 is a transmembrane glycoprotein that binds to many extracellular matrix proteins, of which hyaluronic acid is the most common. Hyaluronic acid is a key component outside the cell that aids in the control and regulation of cell adhesion, migration, and invasive proliferation. Further, the interaction between hyaluronic acid and osteopontin is believed to lead to tumor progression (26,27). High levels of CD44 mRNA and protein expression levels in breast cancer has been linked to significantly worse overall survival (28). Additionally, elevated levels of CD44 expression was found in tumor-forming cells in numerous cancers (29). Clearly, CD44 is believed to be a valid biomarker for CSCs (30). The absence of CD24, another extracellular glycoprotein, has been shown to increase tumor growth and promote metastasis (31). CD133 has been used in combination with the CD44+/CD24– phenotype to isolate BCSCs (32). Interestingly, expression of aldehyde dehydrogenase 1 (ALDH1), an intracellular enzyme that oxidizes aldehydes and retinol, is considered one of the top markers for CSCs in the breast and has been shown to illicit remarkable treatment resistance, a more aggressive phenotype, and ultimately poorer outcomes in patients (33). Despite differences among different subtypes of breast cancer, positive ALDH1 expression has been shown in significantly large proportions compared to other CSC-related markers (34). Although numerous studies have contributed to a better understanding of BCSC surface markers, the picture is still not fully understood. It is often observed that CSCs do not express the same surface markers, or that these markers are not exclusive to CSC and are also variably expressed in cells throughout the breast and body. As a consequence, isolation of BCSCs has been challenging. Currently, there exist no standardized criteria in place to identify BCSCs in human breast cancer (35).

BCSC signaling

Signaling pathways are essential for the regulation of normal stem cells. Many of these pathways are deregulated in CSCs which induce tumor formation. Most notable among these pathways are the Notch, Wnt/Frizzled/β-catenin, Hippo, and Hedgehog signaling cascades which are responsible for the formation of CSCs (36-38).

Notch signaling plays an essential role in normal stem cell maintenance and differentiation. Dysfunction of the Notch pathway has been linked to the development of breast cancer and is believed to be upregulated in a variety of cancers (39-42). The Notch pathways are composed of transmembrane receptors (Notch 1–4) which undergo cleavage, nuclear translocation, and subsequent gene activation upon binding Notch ligands. Notch activation via a constitutively active Notch receptor in normal epithelial cells has been shown to induce hyper-proliferation and breast tumor formation (43,44). Therapeutic resistance in breast CSCs is also believed to be associated with Notch signaling and has been an area of strong interest in cancer research (45).

Hippo signaling is a well-established in tissue homeostasis and tumorigenesis. Hippo signaling is modulated via two pairs of kinases, Mst1/2 and Lats1/2. Upon phosphorylation of downstream Yes-associated protein 1 (YAP1) or Lats1/2-induced TAZ transcription is inactivated and leads to cellular degradation, whereas, dephosphorylation leads to YAP/TAZ nuclear translocation and subsequent activation of transcription (46). Abnormal regulation of Hippo pathway leading to overexpression of YAP1 or TAZ has been shown to be elevated in numerous types of cancers and can directly promote tumorigenesis in mouse models (47). Further, metastatic breast tumors have been associated with BCSCs which express a remarkable TAZ abundance further suggesting the significance of YAP/TAZ in CSCs (48,49).

The Wnt/Frizzled/β-catenin pathway is an important regulator of normal breast development as well as abnormal tumorigenesis. The Wnt signaling proteins play an important role alongside the Frizzled family of cell surface receptors and the Dishevelled family of phosphoproteins to regulate the proteolytic degradation of β-catenin. β-catenin plays an unequivocal role in gene transcription that is involved in determining cell migration, cytoskeletal activity, cell polarity, and cellular differentiation and the inhibition of β-catenin signaling has been shown to prevent mammary development and cellular proliferation during pregnancy (50,51). Most notably, overexpression of Wnt signaling pathways led to breast tumor formation in transgenic mice and an increased number of progenitor cells in precancerous mammary glands (52,53).

Hedgehog signaling is another critical regulator of cell proliferation, stem cell maintenance, and cell fate, including cell self-renewal (54). The pathway is essential for the proper development of mammary epithelium and its disruption has been linked to human breast cancer (55). Previous studies have illustrated the interaction of hedgehog ligand with the patched (Ptch) receptor of a neighboring cell leading to the release of activated Gli which undergoes nuclear translocation to regulate gene expression. Gli-1 and Ptch-1 illicit regulatory negative feedback on hedgehog signaling which has been observed to be reduced or lost in a significant proportion of breast cancers (56-58). Further, components of hedgehog signaling have been correlated with activation of breast CSCs and high expression levels have also been linked to maintenance of the tumor microenvironment which results in autocrine activation of stroma via endogenous generation of Hedgehog ligands (59,60). Aberrant activation of the Hedgehog effector Gli-1 is linked to increased tumor formation and the development of breast cancers in experimental models (61).

Therapy resistance

It is well established that CSCs utilize multiple lines of self-defense against chemotherapeutic drugs and ionization therapies (62). Despite intensive studies in the past, the mechanisms by which breast tumors become chemoresistant is not fully understood (63). Tumor heterogeneity is a key product of CSCs and a key feature of therapy resistance, especially when specifically targeting CSC surface markers (64).

An overwhelming amount of chemotherapy drugs target cells undergoing proliferation. CSCs are predominantly in a resting G0 phase of the cell cycle. Thus, CSCs are inherently immune to the actions of drugs which rely on an overactive cell cycle (64). Further, CSCs under attack by radiation or chemotoxic agents upregulate IGF (insulin-like growth factor) type 1 receptor and increase secretion of IGF1. In the resting G0 phase, this expression pattern inhibits PI3K-AKT signaling and activates Fox03a slowing the cell cycle and stimulating self-renewal (65).

CSCs utilize ALDH1, a member of the NADP+ dependent super family of enzymes known for the physiological and detoxification mechanism involved

in CSCs self-defense. ALDH1 functions by catalyzing the conversion of aldehyde to carboxylic acids, which accumulates as a result of chemotherapy, radiation, or other sources of oxidative stress (66). Downregulating ALDH1, by retinoic acid, has been shown to be an effective treatment in some cancers and a promising treatment sensitizing agent in solid mass breast tumors (67).

ABC transporter activation of ATP-dependent chemotoxin efflux is another mechanism CSCs employ to establish resistance against chemotherapeutic agents and other molecularly targeted therapies (68). Thus, targeting ABC transporters poses a potential mechanism to re-sensitize CSCs and inhibit this pathway of therapy resistance. However, ABC transporters play an important role in normal tissue physiology and their inhibition could lead to severe side effects (67).

Current approaches

Eradicating breast cancer is only possible if we overcome the challenges of effectively and specifically targeting breast CSCs. Despite their abundance, the majority of CSC markers are inadequate for targeting as they are also expressed on normal stem cells. CD44 is the most common CSC marker and is a major contributor to stemness (69). Despite numerous challenges associated with CD44 splicing and post-translational modification, anti-CD44 antibodies have been effective at inducing terminal differentiation of CSC resulting in reduced tumor growth and a significant decrease in metastasis (70,71).

Another key CSC marker is CD133 and treatment with a cytotoxic anti-CD133 antibody has proven effective at eradicating numerous cancers *in vivo* (72). Despite the effectiveness of this approach, targeting CD133 is a rather controversial strategy as its function in normal tissues is not yet fully understood. However, bi-specific antibodies have recently been developed to initiate a T cell response against CD133 (73).

Targeting CSC signaling pathways that play critical roles in self-renewal and defense has been an area of increasing research and clinical trials (74). The Notch pathway has been implicated particularly in breast CSCs and is thought to increase the rate of epithelial-mesenchymal transition ultimately contributing to an increase in metastasis of the CSCs (75). Numerous studies have shown inhibition of Notch signaling to resensitize BCSCs to chemotherapeutic agents and radiation therapy (76). In particular, Psoralidin, a plant-based inhibitor of Notch signaling has been shown to effectively decrease bulk tumor size, upregulate pro-apoptotic genes, and inhibit CSC proliferation and self-renewal (77).

Mediation of the Hippo signaling protein YAP/TAZ has been implicated as an important regulator and inhibitor of self-renewal in BCSCs (49). Overexpression of TAZ promoted tumor growth and an increase in the CSC phenotype, whereas, TAZ knockdown models reported a decrease in overall tumor size and significant decrease in CSC proliferation (78). These findings have indicated YAP/TAZ as an important target for the development of cancer therapies.

Dysregulation of the Hedgehog signaling pathway is believed to play a critical role in the formation of CSCs. Cyclopamine, a well-known Hedgehog antagonist used heavily to study tumor behavior, has been shown to deplete CSC populations via inhibition of CSC proliferation, and ultimately result in a decrease of the overall tumor size in multiple cancers (79-82). Currently, the most direct and potent inhibitor of SMO, a Hedgehog ligand, is vismodegib (83). However, its efficacy in treating breast cancer is not yet clear.

Hedgehog abnormalities are linked to dysfunction of the Wnt pathway which plays a role in maintaining the self-renewal capabilities of CSCs. Wnt/Frizzled/β-catenin inhibitors include non-steroidal anti-inflammatories (NSAIDs), COX-2 inhibitors, and glitazone anti-diabetic agents which have all shown promise pre-clinically as therapy agents capable of reducing the ability of CSC to self-renew (83,84). Additionally, anti-Frizzled receptor antibodies have proven effective at reducing tumor growth and regressing CSC populations (85). However, their use is not believed to be safe considering the importance of the Wnt pathway in normal tissue homeostasis (86).

In addition to targeting CSC surface markers, transporters, and signaling pathways, many studies have demonstrated decreased tumor growth by targeting the tumor microenvironment resulting in an increase in the effectiveness of chemotherapy (87,88). In particular, repertaxin, a non-competitive inhibitor of IL-8 cytokine is one example of such a drug proven to effectively target human BCSCs (89). Lastly, recent studies have demonstrated the use of cannabinoid receptor agonist, ACEA, as an effective agent to decrease the invasiveness of BCSCs (90).

Conclusions

There is compelling evidence that cancer is a disease manifested and maintained by stem cells. Breast cancer

remains a major cause of morbidity and mortality in women worldwide. While tremendous amounts of research have been done to understand breast cancer, there is still much we do not fully understand. We have learned that CSCs are responsible for tumor initiation, development, metastasis, and most importantly recurrence after treatment. We have attempted to provide a representative overview of breast cancer prevalence, the stem of its manifestation, and novel therapies currently being explored to treat patients with this relentless disease.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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