Review Article

Are breast cancer stem cells the key to resolving clinical issues in breast cancer therapy?

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Abstract: Despite the dramatic advances in breast cancer treatment over the past two decades, it is still the most common malignancies in women. One of the reasons patients succumb to breast cancer is treatment resistance leading to metastasis and recurrence. Recently, cancer stem cells (CSCs) have been suggested as a cause of metastasis and recurrence in several cancers because of their unique characteristics, including self-renewal, pluripotency, and high proliferative ability. Increasing evidence has implicated breast cancer stem cells (BCSCs) as essential for tumor development, progression, recurrence, and treatment resistance. BCSCs exhibit resistance to treatment owing to several inter-related factors, including overexpression of ATP-binding cassette (ABC) transporters and increased aldehyde dehydrogenase (ALDH) activity, DNA repair, and reactive oxygen species (ROS) scavenging. In addition, the Notch, Hedgehog, and Wnt signaling pathways have been suggested as the major pathways involved in the self-renewal and differentiation of BCSCs. Despite growing evidence suggesting the importance of BCSCs in progression and metastasis, clear criteria for the identification of BCSCs in clinical practice have yet to be established. Several potential markers have been suggested, including $CD44^{+}/CD24^{-/low}$, ALDH1, EpCAM/ESA, and nestin; however, there is no standard method to detect BCSCs. Triple-negative breast cancer, which shows initial chemosensitivity, demonstrates worsened prognosis due to therapy resistance, which might be related to the presence of BCSCs. Several clinical trials aimed at the identification of BCSCs or the development of BCSC-targeted therapy are in progress. Determining the clinical relevance of BCSCs may provide clues for overcoming therapy resistance in breast cancer.

Keywords: Breast cancer; cancer stem cell (CSC); therapy resistance; aldehyde dehydrogenase 1 (ALDH1)

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Introduction

Breast cancer is the most common malignancy in women in most of the world (1). Despite recent improvement in the 5-year survival rate due to treatment advances, more than 40,000 women in the US die from breast cancer every year. For many malignancies, cancer stem cells (CSCs) have been implicated in the metastasis and recurrence due to treatment resistance (2). CSCs are a small population of cells in the tumor that have unique characteristics, such as self-renewal and the ability to generate heterogenic lineages of cancer cells (3). These characteristics make CSCs a likely source of tumor initiation, heterogeneity, progression, metastasis, and recurrence (4). In numerous solid tumors, including those in brain, pancreatic, ovarian, and breast cancers, CSCs show

resistance to chemotherapy and radiotherapy. Furthermore, CSCs exhibit characteristics of epithelial-to-mesenchymal-transition (EMT), a known mechanism of metastasis (5). Although several biomarkers have been developed for CSCs, CSC characterization is not currently part of standard clinical practice. In this review, we will discuss our current understanding of the characteristics and biomarkers of CSCs in breast cancer and describe H19, a new novel potential biomarker of breast cancer stem cells (BCSCs).

BCSCs

The origin and characteristics of BCSCs

Modern stem cell theory was developed from studies on hematological malignancies in the 1960s, and BCSCs were first isolated from solid tumors in 2003. The tumorigenicity of BCSCs was suggested by their phenotype: $CD44^{+}CD24^{low/-}$ and lack of lineage markers, such as CD2, CD3, CD10, CD16, CD18, CD31, CD64, and CD104b (6). Several theories have been proposed for the origin of BCSCs, including improper regulation or mutations that may lead to the transformation of dormant normal stem cells into BCSCs (7), *de novo* misplacement of somatic stem cells (8), and intratumoral lineages differentiated from common progenitor cells (9).

Common BCSC characteristics have been reported in several studies. BCSCs have the ability for self-renewal and high proliferation and are able to generate heterogenic lineages of cancer cells, so called "pluripotency." These BCSC characteristics are recognized as one of the reasons for treatment failure.

Therapy resistance

Treatment modalities for breast cancer include surgery, chemotherapy, endocrine therapy, and radiotherapy. One of the clinical problems with BCSCs is their resistance to current chemotherapy, endocrine therapy, and radiotherapy. There are four major mechanisms that lead to treatment resistance in BCSCs: overexpression of ATP-binding cassette (ABC) transporters, increased aldehyde dehydrogenase (ALDH) activity, increased DNA repair, and elevated reactive oxygen species (ROS) scavenging.

ABC transporters are transmembrane proteins that can pump various compounds and small molecules from the cell. These transporters are considered important in multidrug resistance in various cancers (10). Among the 49 known ABC transporters, ABCB1 [P-glycoprotein (PgP), multidrug resistance protein 1 (MDR1)], ABCC1 [multidrug resistance-associated protein 1 (MRP1)], and ABCG2 [breast cancer resistance protein (BCRP)] have been shown to be related to multidrug resistance in breast cancer, because these transporters pump out anthracycline or taxans, two key drugs for breast cancer treatment (11). Furthermore, Jonker *et al*. demonstrated that ABCB1 and ABCG2 contribute to the stem cell phenotype in normal murine mammary glands (12). Britton *et al*. demonstrated that the CSCs present in breast cancer cell lines show increased expression of ABCG2 and increased drug resistance (13,14). These results suggest the important role of ABCB1 and ABCG2 in BCSCs.

ALDH is a family of enzymes involved in the oxidation of intracellular aldehydes to carboxylic acids and retinoic acid and in γ-amino butyric acid biosynthesis, and ALDH plays a significant role in the survival and differentiation of BCSCs (15,16). ALDH induces radioresistance in BCSCs both through direct removal of oxygen radicals and indirect production of the antioxidant compound nicotinamide adenine dinucleotide (phosphate) (17). ALDH1 is also associated with breast cancer malignancy, metastasis, and invasion (18). We reported that ALDH1 expression in breast cancer is related to aggressive phenotypes and poor prognosis (19). ALDH1-positive tumors exhibit paclitaxel and epirubicin resistance, and the population of ALDH1-positive cells was shown to increase after chemotherapy (20).

Increased DNA repair is another mechanism of therapy resistance. Radiation and several chemotherapy drugs damage DNA by inhibiting DNA synthesis or topoisomerase activity, or by promoting the formation of DNA crosslinks. Double strand breaks are the most lethal type of DNA damage, which is critical for cancer cells. DNA repair failure usually results in cell death (21); however, BCSCs can repair this lethal type of DNA damage two ways, through homologous recombination (HR) or non-homologous end-joining (NHEJ). HR is similar to chromosomal crossover during meiosis; it requires the presence of a nearly identical sequence, and sister chromatids are used as a template for repair of the break. Although HR can be used as a repair mechanism only during S to G2 phase after DNA replication, (owing to the requirement for a sister chromatid), HR introduces fewer errors than NHEJ. However, in NHEJ, the ends of a double-strand break are directly ligated without the need for a homologous template, and repair via NHEJ can occur in G1 phase; however, it is

associated with increased errors compared to HR (22).

ROS are chemically reactive molecules containing oxygen that are formed as a natural byproduct of normal oxygen metabolism. At normal levels, ROS participate in the regulation of various physiological events, such as cell proliferation, cell migration, wound healing, and angiogenesis (22). However, excess ROS produced in response to environmental stresses, such as radiation, UV, or heat exposure, interact with cell components, such as DNA, proteins, and lipids, which typically induces cell death (23). However, CSCs have specific mechanisms to guard against the genotoxic effects of ROS, including effective ROS scavenging and lower levels of ROS production. In addition, the genes encoding superoxide dismutase, catalase, and glutathione peroxidase, all of which are involved in ROS scavenging, are significantly upregulated in BCSCs (24). These four mechanisms of therapy resistance in BCSCs are correlated with each other.

Identification of BCSC markers

BCSCs were first identified and isolated according to their $CD44^+/CD24^{-/low}$ Lin^- phenotype in 2003 (6). Since then, the $CD44^+/CD24^-$ phenotype has been used as a reliable phenotype for the isolation of BCSCs (25-27). 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 element for cell adhesion, motion, migration, proliferation, invasion, and angiogenesis (28), and its interaction with osteopontin leads to tumor progression (29). In contrast, the absence of CD24, another small surface glycoprotein, enhances tumor growth and metastasis (30). Higher levels of CD44 mRNA and protein expression were observed in the basal subtype of breast cancer, which has a poor clinical outcome (31). Patients with tumors overexpressing CD44 have significantly worse overall survival. Whole genome analysis revealed that CD44 expression was enriched in basal-type breast cancer and was correlated with EMT and CSC gene profiles (31). Another recent study showed that CD44 expression was elevated in tumor-initiating cells in many cancers (32). Thus, CD44 is thought to be a biomarker for CSCs (33). Subsequent functional studies have shown that CD44 is involved in tumorigenesis and metastasis in breast cancers (34-36) and many other cancers, such as colon (37-39), bladder (40), and gastric (41) cancer.

As mentioned above, ALDH is a recognized BCSC marker (42). ALDH is a family of cytosolic enzymes that oxidizes intracellular aldehydes and retinol during the differentiation of rudimentary stem cells (43). ALDH1, which is the dominant enzyme in mammals, mediates the conversion of retinaldehyde to retinoic acid (44). Similar to CD44, ALDH1 expression and activity is increased in normal tissue and in many malignancies. ALDH1 also appears to have increased activity in BCSC populations (45).

Intriguingly, studies have suggested that the ALDH1-active cell population only slightly overlaps with the $CD44^+CD24^{low/-}$ BCSC population. Although the population of ALDH1-active, $CD44^+CD24l^{ow/-}$ cells was very small, tumorigenic capacity of this population was highly enriched (42).

Other markers that have been used to identify BCSCs include EpCAM/ESA, nestin, ganglioside GD2, CD133 (prominin-1), CD61/β3 integrin, $CD24^{hi}/CD49F^{hi}/DNER^{hi}$, $CD24^+CD29^+$, Sca1, $CD44^+$ $CD49f^{hi}$ $CD133/2^{hi}$, CD49f and CD61, CXCR4, CXCL1, and HMGCS (26,46-49).

Several clinical trials have aimed to characterize BCSCs in both healthy populations and breast cancer patients (45), which have improved our understanding of BCSC markers. However, there are no standard criteria to identify BCSCs in human breast cancer.

H19 gene as a possible BCSC marker

H19 is a genomic imprinted oncofetal gene localized on human chromosome 11p15.5, and its oncogenic role was recently recognized as cancer stemness. *H19* is only expressed from maternal alleles without a protein product, and it accumulates in the human placenta, several fetal tissues, and adult organs, including the mammary gland (50-53). Aberrant expression of *H19* has been observed in breast cancer as well as numerous other solid tumors. Although the number of studies on *H19* is increasing, the role of *H19* remains controversial (51,54). Hao *et al.* reported that overexpression of *H19* lowered the tumorigenic properties of cells, which demonstrates the tumor suppressor activity of *H19* (55). Conversely, several studies have suggested its tumorigenic role in many cancers, including breast cancer (56-63). A relationship between *H19* and cancer stemness has also been suggested. In many cancers, increased expression of *H19* correlates with overexpression of stem cell surface markers (*CD44*, *CD166*, and *TROP2*) and pluripotency transcription factors (*Oct4* and *Sox2*), enhanced sphere-forming capacity, high cellular proliferation, and a high rate of apoptosis (57,61-63). Thus, *H19* has been suggested as a possible marker of BCSCs.

BCSCs and breast cancer molecular subtypes

Breast cancers have been classified into five major molecular subtypes, based on gene-expression profiling data: luminal A, luminal B, human epidermal growth factor 2 (HER2) overexpressing, basal like, and claudin low (64-66). Although these molecular subtypes are not currently used in most clinical settings, owing to the difficulty of use, certain immunohistochemical features can be used to predict these molecular subtypes.

The luminal-A subtype is the most common, and it is characterized by low-grade histological features, positive estrogen receptor (ER) expression, and no HER2 expression. The luminal-B subtype is similar to luminal-A, but has a higher proliferation index and worse prognosis. HER2-overexpressing cancers have a higher histological grade and worse prognosis that has improved dramatically with HER2-targeted therapies. The basal-like subtype is nearly equivalent to immunohistochemical triple-negative breast cancer (TNBC) in that it lacks ER, progesterone receptor (PR), and HER2 expression, and has a poor clinical outcome (67-70). The claudin-low subtype is characterized by decreased expression of genes related to tight junctions and cell-to-cell adhesion. However, it lacks distinct histological features; therefore, it cannot be identified by immunohistochemical methods (64,71).

These subtypes differ not only in their rates of relapse, metastasis, and response to therapies, but also in the proportion of BCSCs. $CD44^{+}/CD24^{-/low}$ BCSCs are generally enriched in undifferentiated subtypes, such as claudin-low, basal-like, and HER2-overexpressing subtypes. $ALDH1^{+}$ BCSCs have a similarly enriched distribution in basal-like and HER2-overexpressing subtypes. Enrichment of stem-like and mesenchymal signatures in the claudin-low subtype was demonstrated by examining gene expression profiles, and such enrichment might enable EMT. This BCSC-like population might cause therapy resistance in breast cancer.

Future directions

Although our understanding of BCSCs has increased dramatically in recent years, we need to translate these basic findings to the clinical setting. BCSCs can explain the existence of a small population of cancer cells in human breast cancer with high therapy resistance, resulting in poor prognosis. Despite initial chemosensitivity, patients with basal-like subtype have a worse prognosis. This paradoxical characteristic of the basal-like subtype might be because of the presence of BCSCs in the tumor that remain after chemotherapy, causing a higher likelihood of relapse. Identification of BCSCs in breast cancer and the development of BCSC-targeted therapies have the potential to improve survival and quality of life of cancer patients.

Actually, several molecular-targeted therapies against BCSCs have already been developed. The Notch, Hedgehog, and Wnt pathways have been suggested as the major pathways in BCSCs (22,72). Dysregulation of the Notch and Hedgehog pathways, which are involved in normal stem cell self-renewal and differentiation, results in a BCSC phenotype in breast cancer (73). The Wnt pathway plays a pivotal role in stem cell self-renewal and preservation of an undifferentiated state (74). However, these pathways also regulate essential functions in normal tissues and stem cells. Since specificity is the key to developing targeted therapies, they are not ideal targets. Several anti-BCSCs drugs targeting these pathways have been developed and enrolled in clinical trials (45). Determining the clinical relevance of BCSCs will give us clues about how to overcome therapy resistance in breast cancer.

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Footnote

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