Review Article

CANCER STEM CELLS IN BREAST CANCER: A REVIEW

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ABSTRACT

This paper is written for those women who are affected from breast cancer almost one in eight women in the western world as affected with breast cancer with a total of about one million new cases per year worldwide; of which 35% will die. Breast canricoma is eventually genetically complex entity with approximately 70% of patients with breast cancer has bone metastasis. They are associated with poor prognosis and the available treatment options are very limited. The new perspective of stem cell therapies have shown enhanced promises in the research aspect of the breast carcinoma management and prognosis.

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Keyword: Breast carcinoma, metastasis, stem cell, prognosis.

INTRODUCTION

Stem cells are undifferentiated cells which can differentiate into specialized cell(1). They serve as internal repair system, dividing limitlessly to replenish other cells as long as the person remains alive. When a stem cell divides, the new cell has the potential either to become a stem cell or become another type of cell with specialized function; examples include a brain cell, muscle cell, or a red blood cell (2).

Stem cells are distinguished from other cell types by two important characteristics(2). First, they are unspecialized cells capable of renewing themselves through cell division, sometimes after long period of inactivity. Secondly, under certain physiologic or experimental conditions, they can be induced to become tissue/organ-specific cells with special functions. In some organs, such as the gut and bone marrow, stem cells regularly divide to repair and replace worn out or damaged tissues. In other organs, however, such as the pancreas and the heart, stem cells only divide under special conditions(2). Given their unique regenerative abilities, stem cells offer new potentials for treating diseases such as diabetes, and heart disease (2).

Stem cells differ from other kinds of cells in the body. All stem cells regardless of their source have three general properties: they are capable of dividing and renewing themselves for long periods; they are unspecialized; and they can give rise to specialized cell types(3).

Until recently, scientists primarily worked with two kinds of stem cells from animals and humans: embryonic stem cells and non-embryonic somatic or adult stem cells(4). The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found(5). Adult stem cells have been identified in many organs and tissues, including brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium and testis. They are thought to reside in a specific area of each tissue (called a "stem cell niche") (5).

The detailed study of the biology of mouse stem cells led to the discovery, in 1998, of a method to derive stem cells from human embryos and grow the cells in the laboratory. These cells are called human embryonic stem cells. The embryos used in these studies were created for reproductive purposes through in-vitro fertilization procedures.

In 2006, researchers made another breakthrough by identifying conditions that would allow some specialized adult cells to be "reprogrammed" genetically to assume a stem cell-like state. This new type of stem cell, called induced pluripotent stem cells(1). These are adult cells that have been genetically reprogrammed to an embryonic stem cell-like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells(6).

BREAST CANCER STEM CELLS

Breast cancer affects almost one in eight women in the Western world with a total of about one million new cases per year worldwide; of which 35% will eventually die (7). Breast cancer is a complex and heterogeneous disease with several histological and molecular manifestations within tumors and between patients (7).

The mammary gland is a dynamic organ that goes through significant changes during the menstrual cycle, development, pregnancy, lactation, and involution. Normal mammary gland development and homeostasis is a stem cell-driven process and key signaling pathways have been identified that control these processes. Mounting evidence indicates that the same genes that control physiological organ development and function are often deregulated in cancer (8).

Stem cells are obvious targets of accumulating oncogenic mutations due to their longevity and capacity for indefinite proliferation. It was only in 1990s Dick et al(7).established the existence of tumor initiating cells or Cancer stem cells from myeloid leukemia. By careful immunophenotyping and fractionation of subpopulations of tumor cells, they established that only a fraction of the bulk of the myeloid tumor had the capacity to self-renew *in-vitro* and give rise to transplantable leukemias *in vivo* (9).

CANCER STEM CELLS

Cancer stem cells (CSCs) are cancer cells that possess characteristics associated with normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer sample. Cancer stem cells are therefore tumorigenic(8). Cancer stem cells may generate tumors through the stem cell processes of self-renewal and differentiation into multiple cell types. Such cells are proposed to persist in tumors as a distinct population and cause relapse and metastasis by giving rise to new tumors. Therefore, development of specific therapies targeted at CSCs holds hope for improvement of survival and quality of life of cancer patients, especially for patients with metastatic disease(10).

Normal stem cells that acquire mutations during tumor evolution continue to exist within tumors and are responsible for the initiation and maintenance of neoplastic growth. Tumor initiator cells retain key stem cell properties such as self-renewal and the capacity to generate progenitor cells, in contrast with the bulk of tumor cells. There is increasing evidence that tumor initiator cells are enriched in breast cancer patients after conventional treatment, indicating their intrinsic therapeutic resistance(11).

CANCER STEM CELLS IN BREAST CANCER

Mammary development in humans starts as a primary ectodermal outgrowth in 4-6 months old embryo. At this stage, the primary bud contains a central and a peripheral-basal cell population, which will give rise to different cell layers (12).

The epithelial buds start to form from the primary bud in the 21-25 weeks of embryonic age. While some individuals have a few branched ducts at that point of development, highly structured ductal tree together with regular lobules as it is observed in adults are also observed. After birth, the effect of maternal hormones lessens and the newborn's breast involutes. In females, the ductal tree development and the stromal enlargement continue further only during puberty(13).

Pathways involved in patterning and morphogenesis during mammary gland development are also implicated in breast cancer formation. Among these are neuregulin 3 (NRG3) (an epidermal growth factor receptor (EGFR) ligand involved in placode induction), Wnt signaling (essential for midline specification), fibroblast growth factor (FGF) (critical for inductive signaling to the placode) and Notch signaling important in luminal cell fate commitment which are all frequently activated/mutated in human breast cancer (14).

The risk of developing breast cancer is one in every eight women in the western world and it increases with age. This is thought to be related to the accumulation of multiple mutations in cells of the mammary gland over lifetime of women. Damaged stem cells with unlimited potential of proliferation can give rise to delayed cancers such as breast cancer after having a normal phenotype for decades. This is important because once it becomes possible to identify such cells; approaches to eliminate them would decrease the risk of breast cancer incidence and recurrence(15).

The CSC hypothesis states that, although CSCs represent a rare population of cells within a tumor, their high tumorigenic capacity drives tumorigenesis. Due to their intrinsic stem cell-like properties, CSC proliferation generates more CSCs, and all the differentiated cell types that compose the bulk of the tumor. Non-CSCs in the tumor have been shown to proliferate at a faster rate than CSCs, but have little tumor-initiating potential. Because CSCs exhibit increased resistance to toxic and chemical insults, this specific subpopulation of cells is believed to underlie resistance to chemotherapy and disease relapse. In fact, the CSC model points that all CSCs must be eradicated to eliminate a tumor and prevent its recurrence(16).

In 1994, the acute myelogenous leukemia stem cell (LSC) was identified and characterized. Since then, despite accumulating evidence that LSCs are responsible for maintenance and transfer of blood cancers, researchers doubted the existence of an analogous cell type in solid tumors. But in 2003 an influential report describing the prospective identification of human breast cancer stem cells changed the landscape of breast cancer research(17).

Using human breast tumor samples, which were xenografted into the mammary glands of immunodeficient mice, the investigators reported that a small population of CD44$^+$/CD24$^-$/Lin$^-$ human breast cancer cells were enriched for tumorigenic potential. Thus, most researches now consider that, like leukemia, solid tumors such as prostate, breast, colon, brain, and pancreatic cancers contain a small fraction of self-renewing tumorigenic cells that give rise to and maintain the bulk of the tumor mass(18).

BREAST CANCER STEM CELL MARKERS AND THEIR USES

ALDH-1	GLI-1
BMI-1	GLI-2
CD24	IL-1 alpha/IL-1F1
CD44	IL-6 R alpha
Connexin 43/GJA1	CXCR1/IL-8 RA
CXCR4	Integrin alpha 6/CD49f
DLL4	PON1
EpCAM/TROP1	PTEN
ErbB2/Her2	

The first prospective identification of breast cancer stem cells from human breast cancer specimens reported that within lineage - (tumor cells depleted with expression of normal antigens CD2, CD3, CD10, CD16, CD18, CD31, CD64, and CD140b) population up to 35 % of cells displayed a CD44$^+$CD24$^{-/low}$ marker profile that was able to efficiently generate transplantable tumors in mice. Interestingly, ALDH1$^+$phenotype within a number of human breast tumor samples defined a subpopulation of cancer stem cells not overlapping with the EpCAM$^+$ CD44$^+$ CD24$^-$ phenotype . When the cells were isolated based on ALDH1$^+$ EpCAM$^+$ CD44$^+$ CD24$^-$ phenotype, this population was highly enriched with TIC.

ALDH-Aldehyde dehydrogenases (ALDHs) are NAD(P)$^+$-dependent enzymes that detoxify aldehydes by oxidizing them to carboxylic acids. A total of 19 ALDHs are present in humans, expressed in a variety of organelles and having different substrate preferences(19). Studies have shown that ALDH1 expression did not differ between BRCA1 (breast cancer 1) mutation carriers (who are at increased risk of developing breast cancer) and non-carriers. Others however have shown a close correlation between BRCA1-deficiency and ALDH1 FLUOR positivity in clinical samples and experimental models of BRCA1-related cancer. Even though, numerous studies have proposed the existence of breast tumor initiator cells, to date the exact nature of these cells remain undefined and far from clinical utility(20).

BMI-1- The BMI-1 polycomb ring finger oncogene, a transcriptional repressor belonging to the Polycomb group of proteins plays an important role in the regulation of stem cell self-renewal and is elevated in several cancers(21). BMI-1 is overexpressed in several high-grade, invasive ductal breast adenocarcinomas, thus supporting its role as a prognostic marker. While BMI-1 overexpression increased self-renewal and promoted epithelial to mesenchymal transition , its knockdown reversed epithelial to mesenchymal transition , reduced stemness, and rendered cells drug sensitive, thus highlighting a crucial role for BMI-1 in regulating the stemness and drug response of breast cancer cells(22). Few studies also found a correlation between BMI-1 expression and lymph node metastasis in breast cancer(23-24) suggesting a role for BMI-1 in cancer metastasis(25).

CD24- also known as heat stable antigen(26)
Cd24 is a small cell surface protein molecule anchored by glycosyl-phosphotidyl-inositol in a wide variety of cancer cells(27).It is heavily glycosylated and functions in cell-cell and cell-matrix interactions(27-29). CD24 was discovered in mice as a heat-stable antigen and was used as a marker to differentiate hematopoietic cells and neuronal cells (30-31).

CD24 is highly expressed in ovarian, breast, prostate, bladder, renal, nonsmall cell carcinomas, and other human cancers(28-32). It is involved in cell adhesion and metastasis. This indicates that CD24 could be a significant marker in tumour prognosis and diagnosis. Functionally, it is identified as an alternate ligand for P-selectin, an adhesion receptor on platelets and endothelial cells(33), through which their interaction facilitates the passage of tumor cells in blood stream during metastasis. It increases proliferation and adhesion of tumor cells to fibronectin, collagen, and

lamin(32). The metastatic associations of CD24 increase its importance as a prognostic factor and a new CSC marker high expression of CD24 is involved in tumour progression(27)and metastasis(34).

CD44-CD44 is a multifunctional class I transmembrane glycoprotein(35) generally acts as a specific receptor for hyaluronic acid, promoting migration in normal cells and highly expressed in almost every cancer cell in its standard or variant form(36). CD44 is extensively used as a surface marker for isolating CSCs from breast, prostate, pancreas, ovarian, and colorectal cancers (37-38). As few as 100 cells, $CD44^+$ cells promoted tumorigenesis in breast, and colorectal cancer displaying stem cell properties such as self-renewal and differentiation. It has been suggested that CD44 is an important molecule for metastasis because a nonmetastatic rat glioma cell line acquired metastatic properties when a splice variant of CD44 was ectopically over-expressed. In addition, CD44 variant isoforms are differentially expressed during pregnancy and involution, indicating a role in normal breast epithelial homeostasis.

Connexin 43 is a 43 kDa member of the alpha-type subfamily within the connexin family of transmembrane proteins. It is the most common type of connexin in cardiac muscle cells, and also occurs in hepatocytes, astrocytes and ovary granulosa cells. Human and rat Cx43 are 98% aa identical over the entire length of the molecule(39).

CXCR4, also known as fusin or LESTR, is a G protein-coupled receptor member of the CXC subfamily of chemokine receptors. Human CXCR4 is 352 amino acids in length with a predicted molecular weight of 40 kDa. The mouse and rat proteins share 91% sequence identity with the human protein. CXCR4 is the chemokine receptor for CXCL12. CXCR4 is a critical regulator of progenitor and stem cell mobilization and recruitment during development and during hematopoiesis. Ever since the role of CXCR4 in breast cancer metastasis was first documented in 2001(10). This field has moved forward significantly. Many studies have been conducted which confirms conclusively that increased abundance of CXCR4 in breast cancer cells is associated with enhanced metastatic potential. In 2011, Yagi et. al; reported that CXCR4 is required for the migration of breast cancer cells from the primary site through the basement membrane. It is also implicated in trans-endothelial migration via the activation of the small GTPase Rho, through the heterotrimeric G-proteins associated with it. CXCL12 acts through CXCR4 to stimulate migration and intravasation of the breast cancer cells. Interestingly, recent reports suggest that hypoxic

conditions induce tumor cell CXCR4 expression along with endothelial CXCL12 expression and stimulate trans-endothelial migration towards a CXCL12 gradient thereby facilitating the initial steps of metastasis (11).

Delta-like ligand 4 (Dll4) is a Notch ligand that is predominantly expressed in the endothelium. Dll4 is expressed in cancer associated endothelial cells, but not the endothelium adjacent to non-lactating normal breast epithelium (12).

ErbB2, also known as Neu and Her2, is a type I membrane glycoprotein that is a member of the ErbB family of tyrosine kinase receptors. ErbB family members serve as receptors for the EGF family of growth factors. Among ErbB family members, ErbB2 is unique, it has no identified ligands. Rather, ErbB2 heterodimerizes with the other ErbB family members (EGFR, ErbB3, and ErbB4) to form higher affinity signaling complexes(40).

The **GLI1** transcription factor is highly expressed in the IBC cell lines. There is a growing body of evidence that shows that the downstream transcription factor GLI1 is an essential marker for hedgehog-pathway activation(13).

Interleukins- Produced by activated macrophages, Interleukin (IL)-1 stimulates thymocyte proliferation by inducing IL-2 release, B-cell maturation and proliferation, and fibroblast growth factor activity. IL-1 proteins are involved in the inflammatory response, being identified as endogenous pyrogens, and are reported to stimulate the release of prostaglandin and collagenase from synovial cells(14).

IL-6-The multifunctional factor IL-6 exerts its activities through binding to a high-affinity receptor complex consisting of two membrane glycoproteins. Chronic low-grade inflammation plays an important role in the pathogenesis of several cancer forms including breast cancer. The pleiotropic cytokine IL-6 is a key player in systemic inflammation, regulating both the inflammatory response and tissue metabolism during acute stimulations(15).

PON (paraoxonase/lactonase) -family members PON1, PON2 and PON3 are calcium-dependent antioxidant enzymes. PON1 is secreted into the bloodstream associated with high-density lipoproteins (HDLs). Serum PON1 concentrations vary widely among normal individuals, in part due to differential expression of some polymorphisms. The antioxidant activity of PON1 and other PON members may slow

the initiation and progression of atherosclerosis(41-42).

The **PTEN** gene is often mutated in primary human tumors and cell lines, but the low rate of somatic PTEN mutation in human breast cancer has led to debate over the role of this tumor suppressor in this disease. The involvement of PTEN in human mammary oncogenesis has been implicated from studies showing that germline PTEN mutation in Cowden disease predisposes to breast cancer, the frequent loss of heterozygosis at the PTEN locus and reduced PTEN protein levels in sporadic breast cancers(16).

For solid tumors, the cell surface markers currently used to identify human cancer stem cells includes CD44, CD133, epithelial surface antigen (ESA) and CD24, either singly or in combination. The CD44$^+$ is correlated positively with colon, breast, prostate and pancreatic cancer initiator cells. Similarly, CD133$^+$ cells have been shown to initiate human glioblastoma, colon, prostate and pancreatic cancers in mice. The phenotype of pancreatic and breast cancer initiator cells and normal breast progenitor cells is ESA$^+$. Finally, CD24 is positively correlated with tumorigenicity in pancreatic cancer but negatively correlated in breast cancer, yet CD24$^+$ cells are associated with invasive breast cancer(21).

CD44, CD24 and ALDH1 are the most consistently used biomarkers to identify and characterize the breast CSC (43).

CONCLUSION

The 'tumor stem cell' theory suggests that a small percentage of cells in a tumor harbors intrinsic characteristics making them resistant to treatment. This could explain how patients with metastatic disease show clinical relapse several months after starting treatment due to the survival of a small group of cells with unique characteristics, including the ability to give rise to a new population of cells with a resistant phenotype(18).

The most widely accepted model for metastasis is the 'seed and soil' hypothesis postulated by Paget. He suggested that malignant tumor cells are shed from the primary tumor and disseminated in the entire body although they will metastasize when the shed (disseminated tumor cells) and soil (secondary organ) are compatible. Subsequently, knowledge in this area has expanded significantly. However, the mechanisms underlying the entire process are still unclear, and currently available therapies are mainly palliative. The tumor stem cell hypothesis suggests that there exists within cancers, a subset of cancer cells that are responsible for tumor recurrence following

chemotherapy and are causative of metastasis. Studies using human cell lines and human tissues suggest that a pattern of cell surface and functional markers define these cancer stem cells(41).

Approximately, 70% of patients with breast cancer have bone metastases. They are associated with poor prognosis and the available treatment options are very limited. Bone metastasis usually presents with severe pain, and these symptoms are usually noted in the femur and pelvic region(17). Of the two types of breast cancer bone metastasis, osteolytic lesions are the most common form and cause destruction of the bone, whereas osteoblastic lesions, which are less common, cause new bone formation. Most patients have components of both bone resorption and bone formation.

The metadata analysis conducted by Zhou et. al; (42) lent support to the cancer stem cell hypothesis by showing a significant correlation between cancer stem cells and common clinical parameters, such as ER, PR, HER2 and tumor grade. Putative stem cell markers, particularly ALDH1, were significantly associated with worse survival.

High-dose chemotherapy with stem cell transplant in breast cancer cases is a way of giving high doses of chemotherapy and replacing blood forming cells destroyed by the cancer treatment.

Because cancer stem cells are resistant to traditional chemotherapy and radiation, we need new notes of treatment that can be targeted directly at these deadly cells.

CONFLICT OF INTEREST
The authors declare that they have no competing interests.

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