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Breast cancer stem cells: Biology and therapeutic implications

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ABSTRACT

Breast cancer remains to be a dreadful disease even with several advancements in radiation and chemotherapies, owing to the drug resistance and tumor relapse caused by breast cancer stem cells. Cancer stem cells are a minute population of cells of solid tumors which show self-renewal and differentiation properties as well as tumorigenic potential. Several signaling pathways including Notch, Hippo, Wnt and Hedgehog and tumor-stroma exchanges play a critical role in the self-renewal and differentiation of cancer stem cells in breast cancer. Cancer stem cells can grow anchorage-independent manner so they disseminate to different parts of the body to form secondary tumors. Cancer stem cells promote angiogenesis by dedifferentiating to endothelial cells as well as secreting proangiogenic and angiogenic factors. Moreover, multidrug resistance genes and drug efflux transporters expressed in breast cancer stem cells confer resistance to various conventional chemotherapeutic drugs. Indeed, these therapies are recognised to enhance the percent of cancer stem cell population in tumors leading to cancer relapse with increased aggressiveness. Hence, devising the therapeutic interventions to target cancer stem cells would be useful in increasing patients' survival rates. In addition, targeting the self-renewal pathways and tumor-stromal cross-talk helps in eradicating this population. Reversal of the cancer stem cell-mediated drug resistance would increase the sensitivity to various conventional drugs for the effective management of breast cancer. In this review, we have discussed the cancer stem cell origin and their involvement in angiogenesis, metastasis and therapy-resistance. We have also summarized different therapeutic approaches to eradicate the same for the successful treatment of breast cancer.

1. Introduction

Breast cancer accounts for the second largest cause of cancer-related mortality among women worldwide. Over past few decades, there has been a significant decrease in mortality rates of breast cancer patients due to advancements in the diagnosis and development of novel radiation and targeted chemotherapies (Siegel et al., 2017). Distinct chemotherapies have been devised based on subtype, gene expression profile and mutational status such as hormonal therapies for hormone receptor-positive luminal A and luminal B subtypes (Rouzier et al., 2005), human epidermal growth factor receptor 2 (Her2) inhibitors for Her2-enriched breast cancer (Nixon et al., 2018), and poly (ADP-ribose) polymerase (PARP) inhibitors for targeting BRCA1-mutant tumors and triple-negative breast cancer (TNBC) (Fong et al., 2009). Even though patients show an initial response to the chemotherapies, many women still experience drug resistance and tumor relapse and, the recurrent form of breast cancer remains to be dreadful and incurable. Ostensibly, drug resistance emerges due to a minute population of cancer cells known as cancer stem cells (CSCs) which show stem-like properties.

Breast cancer stem cells (BCSCs) exhibit the expression of specific molecular signatures such as $CD44^{+}/CD24^{-}$, Aldehyde dehydrogenase 1^{high} ($ALDH1^{high}$), $CD133^{+}$, Ganglioside 2^{+} ($GD2^{+}$), etc. (Li et al., 2017; Liu et al., 2013a). Several pathways are operative in BCSCs to maintain their stemness such as Notch, Hedgehog, Wnt, etc. (Takebe

Abbreviations: SDF-1, stromal cell-derived factor-1; VEGF, vascular endothelial growth factor; OPN, osteopontin; ALDH, aldehyde dehydrogenase; DLL, Delta-like ligands; NICD, Notch intracellular domain; CBF1, C-repeats/DRE binding factor 1; CSL, CBF1/Suppressor of Hairless/LAG1; MMTV, mouse mammary tumor virus; FZD, Frizzled; LRP5/6, low-density receptor-related protein 5/6; HH, Hedgehog; PTCH, Patched; Sufu, Suppressor of fused; RTK, receptor tyrosine kinases; AXL, AXL receptor tyrosine kinase; FAO, fatty Acid β-Oxidation; LKB1, liver kinase B1; ESRP, epithelial splicing regulatory proteins; ANTXR1, anthrax toxin receptor 1; Shk, Shikonin Smo, Smoothened; ABC, ATP-binding cassettes; ER, estrogen receptor; HA, hyaluronic acid; PCI, photochemical internalization; FLU, flubendazole; DNMT, DNA methyltransferase; LSD, lysine-specific demethylase 1

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et al., 2015). Accumulated evidence suggests that hypoxia along with stromal cells such as fibroblasts, macrophages, mesenchymal stem cells (MSCs) and tumor-associated endothelial cells play an imperative role in driving these pathways for enrichment and maintenance of BCSCs (Korkaya et al., 2011). Self-renewal and differentiation are hallmarks of CSCs and it is important for maintaining the heterogeneity of tumor. BCSCs are known to undergo differentiation into endothelial cells to support the formation of new blood vessels, a process termed as vasculogenic mimicry (VM) (Delgado-Bellido et al., 2017). Tumor vascularization is essential for supplying nutrients and O_2 to support vigorously growing tumor. In addition, BCSC-derived endothelial cells might be more resistant to treatment as compared to normal endothelial cells. BCSCs are resistance to anoikis, a programmed cell death that occurs in anchorage-dependent cells (Kim et al., 2012). Hence, these cells migrate through blood circulation and form secondary tumors at distant sites by a process known as metastasis (Economopoulou et al., 2012). BCSCs also promote angiogenesis by secreting various proangiogenic and angiogenic factors like stromal cell-derived factor-1 (SDF-1) and vascular endothelial growth factor (VEGF) (Ping and Bian, 2011). By virtue of their tumorigenic potential and drug resistance phenotype, CSCs have emerged as one of the potential therapeutic targets for breast cancer treatment. In this review, we have discussed the biology of CSCs and their implications in angiogenesis, metastasis and drug resistance. In addition, we have reviewed the different approaches of targeting BCSCs for the diminution of drug resistance and tumor relapse to improve the patients' survival rates.

1.1. Origin of breast cancer stem cells

There are two separable but closely related hypotheses which explain the tumor heterogeneity and origin of BCSCs (Lindeman and Visvader, 2010; Shackleton et al., 2009). According to clonal evolution or stochastic model, all the cells in the tumor have a similar tumorigenic potential and tumor heterogeneity arises as a result of the generation of intra-tumoral clones through the sequential mutations. This model presumes that CSCs can be generated from differentiated mammary cells by virtue of mutations that occur in course of the disease. Exposure to detrimental environmental factors such as radiation and chemotherapies induce genetic alterations in non-malignant somatic cells that prime the *de novo* generation of CSCs by the de-differentiation process (Lindeman and Visvader, 2010). Several reports also suggest that microenvironmental cues induce the malignant transformation of differentiated cells into BCSCs. Hierarchical or CSC model postulates that only a small proportion of tumor cells reside in the tumor has a tumor-propagating potential. These cells exhibit self-renewal properties and are capable of reiterating tumor hierarchy (Fig. 1; Kreso and Dick, 2014; Sin and Lim, 2017). The concept of BCSCs arising from the progenitors/stem cells seems to be more plausible (Kreso and Dick, 2014; Bao et al., 2015). Exhibition of similar phenotypic features and expression of stem cell markers by BCSCs to their lineage-specific normal stem cells supports this hypothesis. For example, mammary stem cells show a $CD44^+/CD24^-$ signature which is also a molecular determinant of BCSCs (Liu et al., 2013a). The BCSC population also shares the specific properties including self-renewal with their lineage-specific normal stem cells or partially differentiated mammary progenitor cells (Kreso and Dick, 2014).

1.2. Breast cancer stem cell markers

Development of CSC-specific biomarkers has facilitated the identification and validation of same *in vitro* and *in vivo* breast cancer models, as well as in patients. The molecular markers which routinely used for identification and validation purposes are CD44, CD24, ALDH1 and CD133 (deBeça et al., 2013). CD44 is a cell surface glycoprotein which is known to play a prominent role in cell signaling, adhesion and migration (Aruffo et al., 1990; Senbanjo and Chellaiah, 2017). Several shreds of evidence suggest that it regulates cancer cell proliferation, angiogenesis, invasion, and metastasis (Senbanjo and Chellaiah, 2017). In recent reports, it has been shown that CD44 interact with hyaluronic acid and/or osteopontin (OPN) to exert various functions like cell survival, invasion, angiogenesis and metastasis (Aruffo et al., 1990; Rangaswami et al., 2006). A recent study has reported that OPN/CD44 signaling axis in the perivascular niche promotes CSC phenotype in glioblastoma (Pietras et al., 2014). Several reports show that tumor or stroma-derived OPN also induces CD44 expression thereby controls CSC phenotype in different types of cancer (Butti et al., 2015).

CD24 is an adhesion glycoprotein expressed on the surface of many cell types and it's a recently discovered ligand for P-selectin (Schäck et al., 2016). The CD24 expression is found in highly differentiated tumor cells (luminol-type) (Kwon et al., 2015). Higher expression of CD44 and lower expression of CD24 marks the CSC population (Li et al., 2017). Combination of CD44 and CD24 expression has been extensively used as a BCSC marker along with epithelial-specific antigen (ESA). As low as 200 $ESA^+/CD44^+/CD24^-$ cells derived from breast tumor were able to form tumors when they administered orthotopically into immunosuppressed mice. However, 100-fold more cells without these markers isolated from the same tumors were not able to form a tumor in the mouse. In addition, it was observed that the tumors generated from $ESA^+/CD44^+/CD24^-$ cells were able to recapitulate heterogeneity of initial tumors (Al-Hajj et al., 2003). Furthermore, implantation of $CD44^+$CD24- cells in immunocompromised mice shows higher bone metastasis (Patanè et al., 2013). A recent report has shown that $CD44^+$/CD24- tumors are associated with poor clinical outcome in ER-ve patients whereas it is associated with longer survival rates in ER + ve patients (Kim et al., 2011). This might be due to higher chemo-resistant property of $CD44^+$/CD24- tumors in ER-ve patients than their counterparts.

ALDH1 is also a marker of BCSCs. It is a detoxifying enzyme that catalyses the oxidation of intracellular aldehydes to carboxylic acids. The activity of ALDH1 in cells is associated with stem cell phenotype and assessed by ALDEFLOUR assay. Hence, ALDH1 is widely used as a marker of BCSCs and its expression is associated with poor clinical outcome in breast cancer. The ALDH1 expression is also linked to drug resistance in breast cancer (Moreb et al., 2012). However, a recent study has reported that $CD44^+/CD24^-$ CSC population is anatomically different from ALDH1 + ve CSCs. Molecular profiling of these populations had shown that the $CD44^+/CD24^-$ sub-population exhibits mesenchymal phenotype with quiescent state whereas ALDH1 + ve subpopulation shows epithelial phenotype with higher proliferative potential (Liu et al., 2013a). CD133 also acts as CSC-specific marker in triple-negative breast cancer (TNBC) and BRCA-1 mutant tumors. Even though specific functions of CD133 have not been established, different splice variants of CD133 are known to interact with cholesterol and thus, have a role in Hedgehog signaling pathway (Liu et al., 2013b). The tumor-initiating cells are also defined by CD49f and CD61 in Her2/neu-induced mammary tumors that developed from the luminal progenitor cells in mice (Lo et al., 2012).

1.3. Cancer stem cells and signaling pathways

Embryonic development and stem cell maintenance are complex and highly regulated processes. Numerous signaling pathways are essential for embryonic development and maintenance of stem cells in adult tissues (Burdon et al., 2002). Several key signaling pathways mainly Notch, Wnt and Hedgehog and their crosstalks dictate the stem cell-specific properties (Katoh, 2007; Takebe et al., 2011). Dysfunction of these stemness signaling pathways moderates self-renewal characteristics thus leads to the detainment of CSC phenotype. Aberrant activation or mutation in stemness-related genes is frequently reported and associated with aggressiveness and cancer relapse (Takebe et al., 2011).

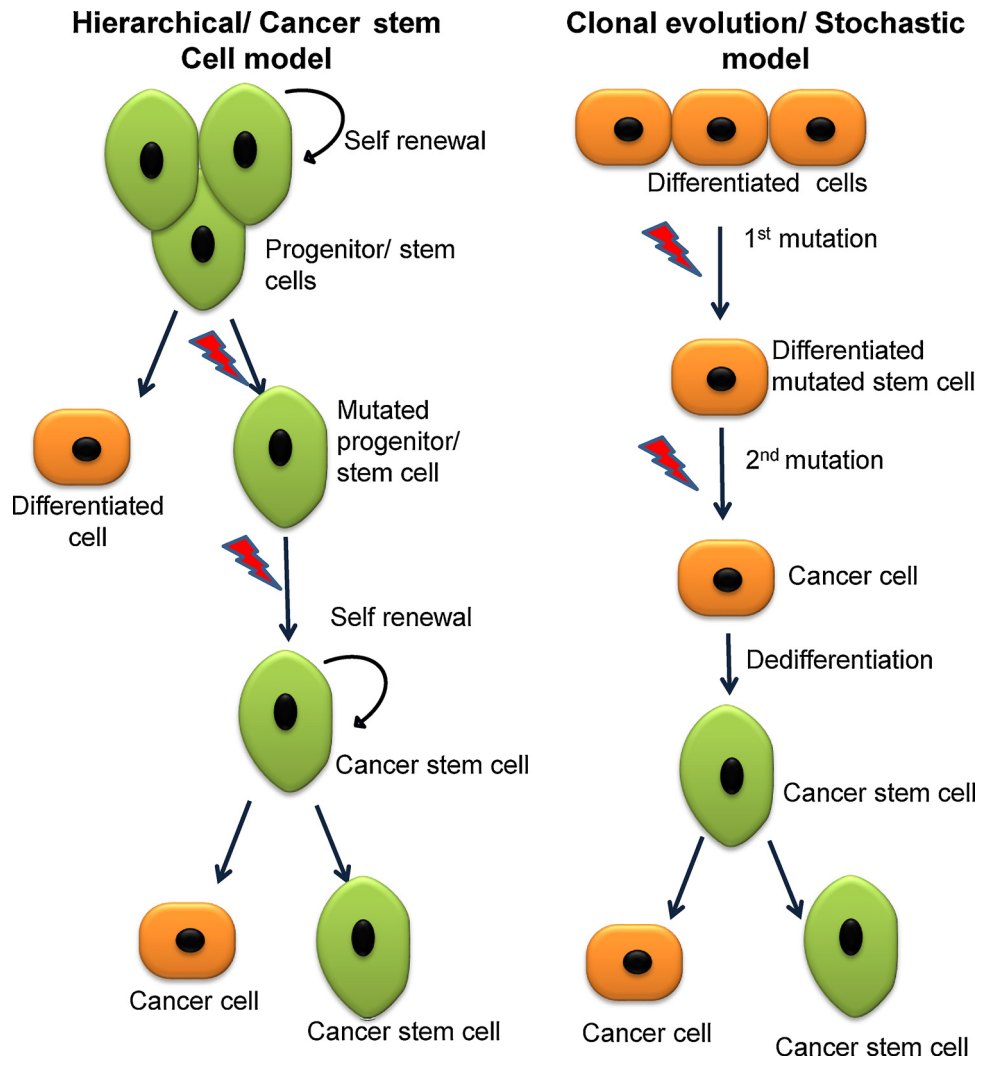

Fig. 1. Origin of tumor heterogeneity and BCSCs. CSC model states that a minute population of cells possesses self-renewal and differentiation capacities, and tumorigenic potentials. These self-renewal and differentiation capabilities contribute to tumor heterogeneity. Clonal evolution model states that BCSCs can be generated from differentiated mammary cells by dedifferentiation process. Tumor heterogeneity ascends as a result of the formation of intra-tumoral clones by the sequential mutations.

2. Notch Signalling in the regulation of breast cancer stem cell phenotype

The Notch is an essential transmembrane signaling receptor, required throughout the embryogenesis and involved in the determination of stem cell fate, cell differentiation, apoptosis and cell cycle progression (Bouras et al., 2008). Notch family consists of Notch1–4 receptors. These are known to bind with five different ligands such as jagged proteins (JAG1 and JAG2) and delta-like ligands (DLL1, DLL3, and DLL4). Both Notch receptors and their ligands are transmembrane proteins expressed in adjacent cells. Ligand-receptor interaction through the cell-cell contact activates Notch signaling. Notch proteins contain extracellular and intracellular domains. Extracellular domain consists of EGF-like repeats which are important for ligand binding. Notch-ligand interaction elicits two successive proteolytic cleavages by ADAM and secretase to release Notch intracellular domain (NICD) (Acar et al., 2016; Chillakuri et al., 2012; Ranganathan et al., 2011). Then, NICD translocates to the nucleus and displaces corepressor protein from CSL (CBF1/Suppressor of Hairless/LAG1)/RBPJ transcription factors that leads to the activation of downstream signaling cascades (Fig. 2; Acar et al., 2016; Bhat et al., 2016). The first possible link between Notch signaling and breast cancer was identified in the mouse mammary tumor virus (MMTV)-induced mouse mammary tumor. Frequent insertion of MMTV between the negative regulatory region (NRR) and transmembrane domain results in constitutive activation of Notch and concomitant release of NICD domain (Dievart et al., 1999; Han et al., 2011; Karamboulas and Ailles, 2013). Moreover, constitutive activation of Notch1 signaling impairs ductal and lobuloalveolar mammary gland development and causes mammary carcinoma in MMTV/Notch1^{intra} Tg (where the Notch1 gene is truncated upstream of the transmembrane domain) mice. A similar phenotype was observed when they did the same experiments with Notch3 (Hu et al., 2006).

In humans, activation of Notch signaling occurs in almost 50% of the breast cancer patients. Expression of Notch signaling components (JAG1, Notch1 and Notch4) is very common in breast cancer and also associated with poor patient's survival. Notch signaling participates in breast tumorigenesis certainly by maintaining BCSC phenotype (Pece et al., 2004; Miele, 2008). Previous studies have clearly shown that CSCs are present in breast cancer cell lines as well as human cancer tissues. Moreover, it has also shown that Notch^{+} (higher activity) and Notch^{-} (lower activity) subsets exist in various breast cancer cell lines including luminal and basal type (D'Angelo et al., 2015). Harrison et al. (2010) have revealed the activation of Notch signaling is higher in enriched CSC population (ESA^{+}/CD44^{+}/CD24^{low}) than in differentiated non-stem cells. Their studies have clearly demonstrated that activation of Notch1 and Notch4 is higher in enriched CSC population

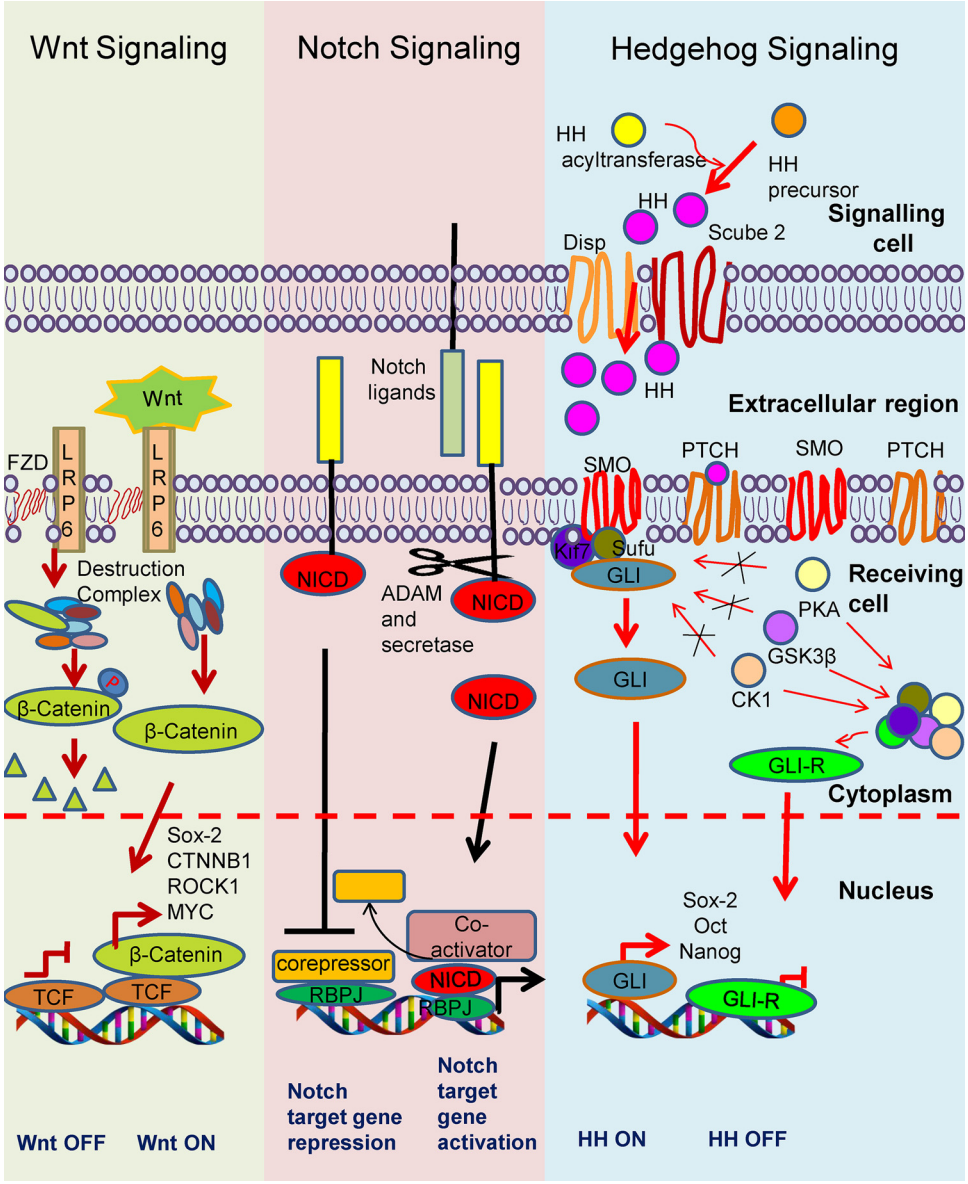

Fig. 2. Multilevel signaling networks in regulation of BCSCs. Activation of Notch, Wnt and Hedgehog signaling aids in maintenance of CSC phenotype, multidrug resistance, angiogenesis, and metastasis. Notch signaling: Notch ligands expressed in signaling cells bind to the Notch extracellular domain present in the signal receiving cells thereby initiate Notch cleavage to generate NICD. NICD is translocated into the nucleus where it interacts with transcription factors to activate notch target genes. Wnt signaling: in the devoid of Wnt ligands, β-catenin gets phosphorylated and proteolytically degraded. When Wnt ligands bind with its receptors (LRP6 –LZD receptors), activated receptors inhibit the GSK-3β-mediated phosphorylation of β-catenin that leads to accumulation of β-catenin in cytoplasm and consequently translocation to the nucleus. Nuclear β-catenin binds with TCF promoter region to activate Wnt target genes. Hedgehog signaling: in the absence of Hedgehog (HH) ligands, PTCH inhibits the translocation of SMO and produces GLI-Repressor through kinase-dependent proteasomal degradation. HH ligand precursor proteins are activated by acyltransferase and translocated to extracellular region. In the presence of HH ligands, the inhibitory action of PTCH on SMO translocation is abolished and GLI protein is translocated into the nucleus to activate HH downstream target genes.

but Notch4 activation is four-fold higher than that of Notch1 activation. Moreover, Notch^{+} cell subset has developed the tumors whereas Notch^{-} cells failed to do so in xenograft mouse model. Higher notch activation in these cells leads to increased sphere formation and higher expression of stemness genes including Oct4, Nanog, Sox2, ALDH and Klf4 (Pannuti et al., 2010). Moreover, an immunohistochemical study of 115 primary breast tissues has shown that Notch expression was strongly associated with the ALDH1A1 expression level. Activation of Notch signaling regulates *ALDH1A1* acetylation through the induction of SIRT 2 expression and activation which is required for tumor-initiating potential (Zhao et al., 2014a). Myeloid-derived suppressor cells (MDSCs) have a key role in the regulation of cancer stemness-associated immunosuppression in breast cancer by the activation of Notch signaling. MDSC-derived nitric oxide (NO) and IL-6 activates Notch signaling and consequently induces STAT3 phosphorylation. The crosstalk between Notch and STAT3 contributes to the maintenance of the CSC pool and supports CSC-mediated metastasis (Peng et al., 2016).

3. Wnt signaling in the maintenance of breast cancer stem cells

Wnt signaling is an evolutionarily conserved pathway which plays a vital role in various physiological and pathological functions including embryonic development, tissue homeostasis and cancer (Wang and Wynshaw-Boris, 2004). Wnt is a glycoprotein that serves as a ligand for Frizzled (FZD), a seven transmembrane serpentine receptor and low-density receptor-related protein 5/6 (LRP5/6) (MacDonald and He, 2012). Nineteen Wnt ligands have been identified so far. Wnt possesses a cysteine-rich conserved motif consists of 350–400 amino acids along with an N-terminal signal peptide for secretion. The interaction between Wnt and its receptor induces two different mechanistic cascades such as canonical or non canonical signaling. In the canonical pathway, binding of Wnt ligands with receptors determines the stability of β-catenin which is the central player of canonical Wnt signaling. In the devoid of Wnt ligands, β-catenin present in cytoplasm undergoes phosphorylation and consequently proteasomal degradation via destruction complex. Destruction complex consists of several proteins such as axin, adenomatous polyposis coli, glycogen synthase kinase 3β, and casein kinase I-α. Binding of Wnt with their cognate receptors decomposes the destruction complex by recruiting intracellular protein, dishevelled. Therefore, levels of non-phosphorylated β-catenin is increased in the cytoplasm that leading to translocation of β-catenin into the nucleus to modulate transcriptional activation of various β-catenin-

TCF/LEF target genes (Fig. 2; Reya and Clevers, 2005; Takebe et al., 2011).

Accumulating evidence suggests that Wnt signaling has a prominent role in orchestrating CSC self-renewal and differentiation. Indeed, aberrant regulation of Wnt increases niche-independent and deviant differentiation of stem cells. Various reports have revealed that expression of Wnt/β-catenin signaling components such as FZD receptors, LRPs, β-catenin, Disheveled, TCF4 and LEF1 have been linked to the poor survival of the patients. Moreover, Wnt signaling is highly active in CSC population ($ALDH^+$ cells) than other tumor cell population ($ALDH^-$ cells) (Cui et al., 2015). Moreover, silencing the Wnt3a reduces the tumor-initiating potential of BCSCs *in vitro*. Increased Wnt signaling has been reported in various subtypes of breast cancer including TNBC. Moreover, TNBC patients which show augmented Wnt signalling, have a high chance of developing lung and brain secondary metastasis. $CD44^+/CD24^-/CD49f^+$ TNBC cells have different metabolic profile than other cancer cells (Pohl et al., 2017). These cells are more dependent on mitochondrial respiration which known to contribute to chemoresistance and DNA damage resistance (Peiris-Pagès et al., 2016).

Several microRNAs have been reported to regulate Wnt signaling and are associated with BCSC phenotype. miR-142 activates the Wnt signaling by decreasing the APC level via recruiting the APC mRNA to the RNA-induced silencing complex (Isobe et al., 2014). Overexpression of miR-374a suppresses the negative regulators of Wnt signaling including PTEN and WIF that eventually enhances Wnt-driven EMT and metastasis in breast cancer cell lines (Cai et al., 2013). Silencing of miR-600 activates the non-canonical Wnt signaling and increases BCSC self-renewal thereby increases the tumorigenicity *in vivo*. miR-600 targets stearoyl desaturase 1 which is an essential enzyme involved in producing active lipid-modified Wnt proteins (El Helou et al., 2017). Down-regulation of Wnt target genes such as Sox2, CTNNB1, ROCK1, and Myc has been observed upon induction of miR-340 in TNBC cell lines. Overexpression of miR-340 decreases the migration, invasion and metastasis (Mohammadi-Yeganeh et al., 2016).

4. Hedgehog signaling in the regulation of Breast cancer stem cell phenotype

Hedgehog (HH) signaling plays an important role in various cellular processes during embryonic development and tissue homeostasis and also a key regulator of cell fate and self-renewal (Briscoe and Thérond, 2013). In mammals, three Hedgehog homologs (ligands) has been reported such as Sonic Hedgehog, Indian Hedgehog and Desert Hedgehog (Scales and de Sauvage, 2009; Takebe et al., 2011). These proteins were synthesized as a precursor protein and further activated by various post-translational modifications including autoproteolytic cleavage, dual lipid modifications, and acylation (by Hedgehog acyltransferase). Binding of these proteins with Dispatched (Disp) and Scube2 protein on the cytoplasmic side of plasma membrane is resulted in discharge of these proteins to extracellular space from signaling cells. Generally, in the absence of HH ligands to the responding cell, an extracellular transmembrane protein, Patched (PTCH) constitutively inhibits the localization of the transmembrane protein, Smoothened (SMO) at the plasma membrane. Consequently, full-length GLI protein is retained in the complex with Sufu (Suppressor of fused) protein and then partially cleaved by the recruitment of PKA, GSK3β, and β-TrCP to generate the GLI transcriptional repressor to inhibit HH target gene expression (Fig. 2; Takebe et al., 2011).

The BCSCs are characterized as $CD44^+/CD24^{-/low}/Lin^-/ALDH1^+$ and these cells exhibit highly active HH signaling to retain the stemness potential. These cells have the potential to develop primary tumor when they are injected into NOD/SCID mice. (Cochrane et al., 2015). Overexpression of GLI is reported in various cancers including breast and it correlates with node-positive, higher grade cancer condition and poor disease-free survival (ten Haaf et al., 2009). Moreover, overexpression of HH signaling components such as SHH, PTCH1, and GLIs has been linked to angiogenesis, ECM degradation, and metastasis (Monkkonen and Lewis, 2017). Stromal activation of HH signaling cascade has been reported in mammary tumor in MMTV-Wnt1 mouse model. HH signaling in the CSCs educates cancer-associated fibroblasts (CAFs) to maintain the suitable microenvironment for existence and survival of CSCs by providing essential cytokines and growth factors. CSCs produce SHH which induces downstream HH signaling cascade in CAFs leads to the release of various ligands including Activin Aand LIF which further supports increased proliferation and ECM decomposition (Valenti et al., 2017). Long noncoding RNAs (lncRNAs) are new players in the regulation of several genes involved in the acquisition and maintenance of EMT-associated cancer cell stemness. The lncRNA-HH activates SHH-GLI1 signaling by targeting GAS1 and increases the expression of Twist, Sox2 and Oct4 to induce EMT in cancer cells to gain stem cell characteristics (Zhou et al., 2016).

5. Other signaling and their crosstalk in the regulation of breast cancer stem cells

Other signaling pathways and their cross-talk including PI3K/Akt/mTOR and JAK/STAT are involved in CSC enrichment and maintenance. Aberrant regulation of individual signaling could result in breast cancer, but these signaling pathways hardly ever drive in isolation. The interplay between these signaling pathways shows the potential to maintain CSC phenotype with the company of external stimuli. Dysregulation of RTK (receptor tyrosine kinases) signaling is a key contributor to breast cancer relapse and drug resistance. Different classes of RTKs including EGFR, PDGFR, VEGFR, AXL, etc., have known to play a major role in breast cancer and share the common downstream signaling cascades which crosstalk with various key signaling involved in the maintenance of BCSCs (Asiedu et al., 2014; Butti et al., 2018). PI3K is one of the important and common downstream molecules for many RTKs which interlink key stemness pathways. AXL is a member of RTKs and its overexpression correlates with tumor stage in breast cancer. Enrichment of AXL signaling is associated with the activation of various signal transduction pathways such as MAPK, NF-κB, STAT, PI3K/Akt. Constitutive activation of AXL has been reported in BCSCs and its known to regulate the expression of CSC associated EMT markers including Snail, Slug, E-cadherin and N-cadherin. Zhang and Grivennikov (2013) have reported that paracrine activation of NF-κB increases the expansion of CSCs via the activation of Notch signaling in breast cancer. Tumor-associated macrophages (TAMs), CAFs and immune cells provide growth factors and cytokines thereby enrich and maintain the CSC population (Fig. 3). Wang et al. (2018) have suggested that leptin-LEPR-JAK-STAT3 signaling activates fatty Acid β-Oxidation (FAO) in BCSCs that is linked to self-renewal and chemoresistance. Activation of FAO might contribute to numerous metabolic advantages to CSCs through increasing ATP production and reducing the accumulation of reactive oxygen species to decrease oxidative stress.

6. Role of cancer stem cells in breast cancer progression

6.1. Role of breast cancer stem cells in promoting metastasis

Metastasis is the dissemination of cancer cells from primary tumor to distant parts of the body. The process of metastasis is tightly regulated by multiple signaling pathways. It is a complex process where cancer cells enter the blood circulation by the process of invasion and move through the circulation to establish a new tumor in distant organs. Increasing evidence suggests that CSCs are responsible for metastasis, because of its inherent anoikis-resistant property. Anoikis is a regulated cell death induced in anchorage-dependent cells when they detach the substratum. Most of the cancer cells die in the circulation whereas CSCs survive and establish metastatic lesions at distant sites

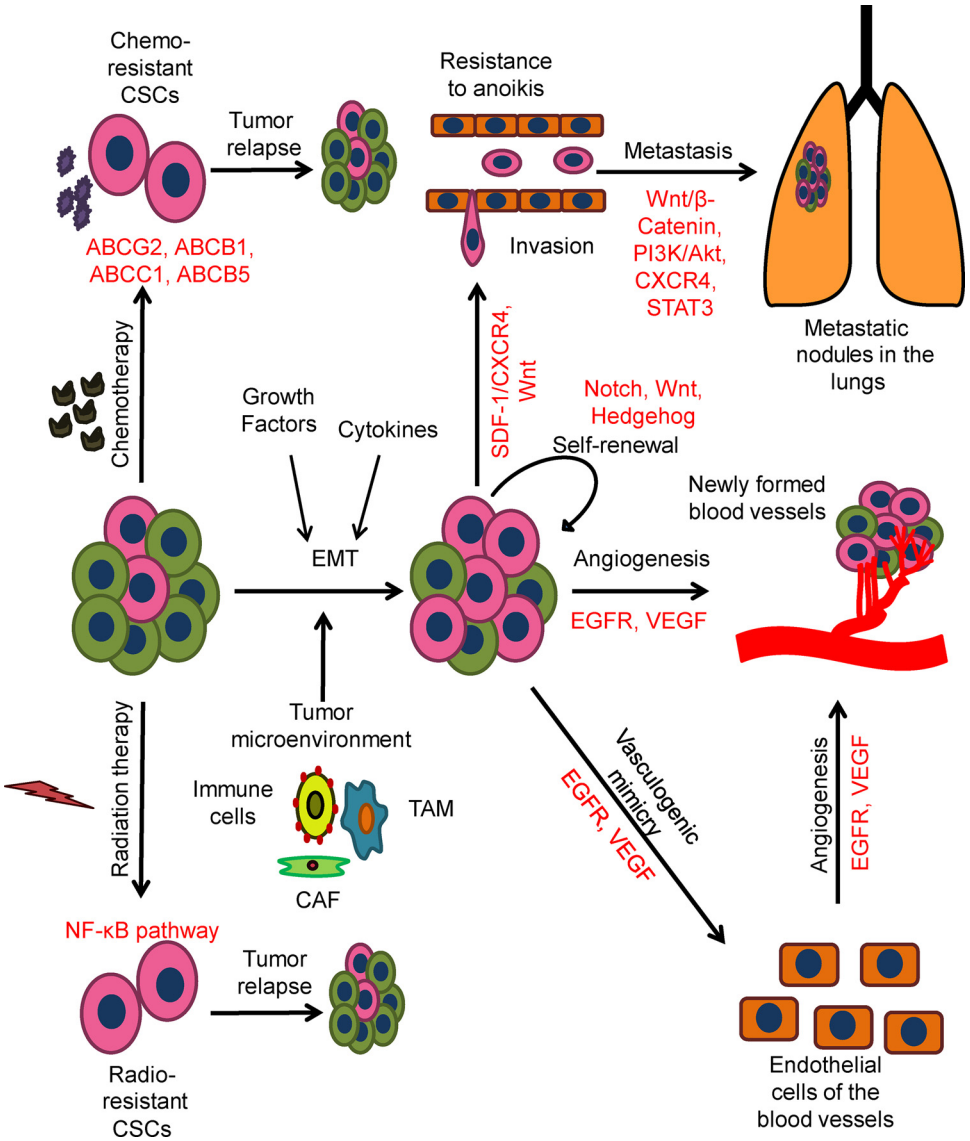

Fig. 3. Role of BCSCs in angiogenesis, metastasis, tumor relapse, and drug and radiation resistance: BCSCs exhibit self-renewal and differentiation properties which help in maintaining tumor bulk. Self-renewal property of CSCs is regulated by both internal and external factors like gene mutations, growth factors, and chemokines that are released by the tumor-stroma. CSCs that survive after chemo and radiation therapies play an important role in tumor relapse. BCSCs survive in blood circulation because of its anoikis-resistant property and play a vital role in tumor cell dissemination. BCSCs are differentiated into endothelial cells by VM and help in tumor angiogenesis. EMT, Epithelial-mesenchymal transition; CAF, Cancer-associated fibroblasts; TAM, Tumor-associated Macrophages.

(Kim et al., 2012, 2016). It was observed that circulating tumor cells (CTCs) show enhanced levels of LKB1 which might help in the survival of these cells (Trapp et al., 2017). BCSCs show higher expression of CXCR4. Stimulation of CXCR4 signaling by SDF-1 induces mammosphere forming capacity and anoikis-resistance in breast cancer cells (Ablett et al., 2014). Wnt activation is also shown to be higher in the anoikis-resistant cell population (Fig. 3; Lamb et al., 2013). Down-regulation of metalloprotease-disintegrin, ADAM12 reduces the cell migration, invasion and anoikis-resistance in claudin-low breast cancer cells by suppressing the activation of EGFR signaling pathway (Duhachek-muggy et al., 2017). Among $CD44^{+}/CD24^{-}$ BCSC subpopulation, the cells which express CD44v variant show higher metastatic ability than that of CD44 s variant-expressing cells. The splicing of CD44 to different variants is mediated by epithelial splicing regulatory proteins (ESRPs). It was also observed that overexpression of ESRP1 in MCF10CA1h cells increases the expression of CD44v and promotes lung metastasis (Hu et al., 2017). Wnt/β-catenin pathway is one of the important pathways involved in regulation of BCSC-mediated metastasis. Survivin is found to be expressed in quiescent-BCSCs and thus, favours the breast cancer metastasis. Survivin helps in self-renewal of CSCs by activating PI3K/Akt-dependant Wnt pathway (Siddharth et al., 2016). It was also found that silencing the BCSC functional biomarker such as anthrax toxin receptor 1 (ANTXR1) by RNAi approach downregulates the Wnt pathway genes and reduces the metastasis in BCSCs (Fig. 3; Chen et al., 2013). Correspondingly, treatment of breast cancer cells with Chinese herbal medicine, Shikonin (Shk) has revealed that STAT3, FAK, and Src are important for metastasis. It was observed that inhibition of STAT3 significantly affected the CSC phenotype as compared to the inhibition of FAK or Src. Combined inhibition of STAT3 and Src or STAT3 and FAK significantly decreases the mammosphere formation, invasion, and migration than inhibition of individual proteins (Thakur et al., 2015). Intrinsic CSCs (iCSCs) present in tumor core aids in metastasis by converting non-stem cells into migratory CSCs (mCSCs). It was reported that mCSCs express CXCR4 and they are responsible for tumor metastasis but not iCSCs (Mukherjee et al., 2016). It was also shown that expression of CD44, CXCR4, and OPN is higher in BCSCs and they might have a role in the metastasis of breast cancer cells to the bone (Ling et al., 2008). Similarly, Smo expression is high in CSCs compared to non-stem cancer cells. It was reported that inhibition of Smo expression by specific siRNA results in downregulation of MMP-2 and MMP-9 in MCF-7 cells suggesting that Smo might play an important role in CSC-mediated metastasis in breast cancer (Wang et al., 2014a, 2014b).

6.2. Role of breast cancer stem cells in vasculogenic mimicry and angiogenesis

Sprouting of blood vessels within the tumor to supply the oxygen and nutrients for continuously growing tumors is called as tumor angiogenesis. Recent studies have found that cisplatin treatment along with carbon beam significantly reduces the angiogenesis and CSC phenotype (Sai et al., 2015). Vasculogenic mimicry (VM) is the process in which aggressive tumor cells form vascular network patterns by its unique plasticity property. Liu et al. (2013b) have found that CD133 expression positively correlates with VM. Later on, it was found that BCSCs which are positive for the expression of ubiquitin-specific protease 44 (USP44) contribute to VM and aggressiveness of breast cancer (Liu et al., 2015). Lee et al. (2014) have observed that ALDH + ve or sphere forming BCSCs can form tube-like structures on matrigel-coated surfaces. EGFR signaling is important for BCSCs to show VM. Inhibition of EGFR signaling by gefitinib or shRNA reduces the VM of BCSCs *in vitro* (Fig. 3). Treatment of breast tumors with anti-angiogenic drug such as bevacizumab induces the hypoxic environment and BCSC phenotype which further promote tumor angiogenesis and aggressiveness. Bevacizumab-induced tumor aggressiveness could be inhibited by targeting CSCs using CRLX101, a nanoparticle conjugated with camptothecin which targets HIF1α (Conley et al., 2015). It is well known that CSCs have the capacity to transdifferentiate into endothelial cells. VEGF treatment induces the expression of endothelial markers in BCSCs. A previous report has shown that miR-27a is upregulated in VEGF-treated BCSCs which further, leading to induction of endothelial properties in BCSCs (Fig. 3; Tang et al., 2014). The above reports clearly suggest that BCSCs might play an important role in tumor angiogenesis; hence targeting BCSCs is required for the better outcome of breast cancer treatment.

6.3. Breast cancer stem cells and chemo- and radiation resistance

Radiotherapy is a standard modality for breast cancer treatment (Baskar et al., 2014) where high energy radiation is used to kill cancer cells by directly causing excessive DNA damage or generation of free radicals which further persuade DNA damage (Balaji et al., 2016). However, Radioresistance remains to be a major challenge for breast cancer treatment. Radioresistance is mainly caused by BCSCs residing in solid tumors. CSCs display resistance to radiotherapy via altered/higher expression of DNA repair enzymes, DNA repair checkpoint proteins and higher activation of free radical scavenging system (Wang, 2015). BCSCs possess higher free radical scavenging capacity due to enhanced expression of components of free radical scavenging system as compared to non-CSCs. This might decrease reactive oxygen species (ROS)-mediated DNA damage and cell death (Diehn et al., 2009; Tothova and Gilliland, 2009). In a previous report, it has been shown that radiotherapy induces the activation of NFκB (Ahmed and Li, 2008). As we know, NFκB plays an imperative role in various pathological and physiological processes as a transcription factor (Morgan and Liu, 2011). In this context, NFκB induces the activation of an array of anti-apoptotic genes such as MnSODandMKP1 which are scavengers of DNA damaging ROS/other free radicals and involved in downregulation of apoptotic signaling pathways. Thus, BCSCs obtain resistance to DNA-damaging radiotherapy via an NFκB-modulated prosurvival signal that leads to increased aggressiveness of recurrent breast cancer (Fig.3; Guo et al., 2003; Karin and Lin, 2002). Her2 (receptor tyrosine kinase) has been considered as a reliable biomarker for CSCs and overexpression of Her2 is associated with increased tumor aggressiveness and relapse, and poor prognosis (Korkaya et al., 2008; Nami and Wang, 2017). Earlier reports have shown that radiotherapy induces the expression of Her2. This indicates that radiotherapy-induced Her2 + ve BCSCs might be responsible for tumor therapy-resistance and relapse with increased aggressiveness (Duru et al., 2012).

BCSCs residing at primary and metastatic sites are accountable for the drug resistance and tumor relapse. Drug resistance is of two types; intrinsic resistance (*de novo* resistance to a broad spectrum of drugs) and acquired resistance (developed as a response to treatment modalities) (Li et al., 2008; Prieto-Vila et al., 2017). BCSCs express drug efflux proteins such as multidrug resistance-associated proteins (MRP), P-glycoprotein and breast cancer resistance protein (BCRP) which are responsible for the resistance to a broad spectrum of drugs (Fig. 3). These ATP-binding cassette (ABC) drug transporters are highly expressed in BCSCs and known to protect these cells from anticancer chemotherapeutic drugs (Leonard et al., 2003; Vasiliou et al., 2009). ABCB1 is also known as P-glycoprotein was found to be overexpressed in 50% of drug resistant-tumors. Human ABCB1 is encoded by the MDR1 gene and functions as an ATP-dependent pump for several hydrophobic compounds including anticancer and antimicrobial drugs (Moitra et al., 2011). ABCG2 is known to function as a homodimer and it shows broad specificity in terms of drug efflux. It has been reported to transport doxorubicin, mitoxantrone, topotecan, methotrexate, tyrosine kinase inhibitors, etc. (Lecerf-Schmidt et al., 2013; Stacy et al., 2013). It has been reported that the percentage of $CD44^+/CD24^-$ cells were increased upon administration of neoadjuvant chemotherapy. In addition, Molecular profiling of chemotherapy-treated tumors closely resembles the $CD44^+/CD24^-$ and mammosphere forming cell features suggesting the effect of chemotherapy on the enrichment of BCSCs (Creighton et al., 2009). CSCs enrichment has been observed in SKBR3 cell-implanted xenograft tumors upon epirubicin treatment (Yu et al., 2007). It was also observed that CSCs play very important role in acquiring resistance to cisplatin and tumor progression in a BRCA1/p53 mammary tumor model (Shafee et al., 2008). Hormone therapy is one of the therapeutic approaches for the management of ER + ve breast cancer. However, 20–40% ER + ve tumors fail to respond to anti-estrogen therapies due to acquired resistance to this therapy. Several mechanisms are responsible for anti-estrogen therapy-resistance. Tumor heterogeneity and BCSCs are main players in the development of acquired resistance to these anti-estrogen drugs. Anti-estrogen therapy evokes tumors plasticity and increases cancer stemness in prolactin-driven ER + ve breast tumors (Shea et al., 2018). It has also reported that miRNAs play a crucial role in CSC-mediated drug resistance (Hu et al., 2018a). Role of different miRNAs in the regulation of stem cell pathways, CSC-maintenance and CSC-mediated drug resistance is listed in Table 1. Roscigno et al. (2017) have shown that miR-24 regulates the BCSC phenotype. Under the hypoxic stress and other toxic stimuli (Drug treatment conditions) expression of miR-24 is upregulated in BCSCs and in turn, it increases the resistance to these factors by downregulating FIH1 and BimL. miR-24 also interferes with chemotherapy-induced apoptosis by suppressing BimL expression. miR-221/222 stimulates BCSC phenotype by activating Akt/NF-κB-mediated COX-2 expression (Li et al., 2016). It has also shown that miR-221/222 regulates drug resistance in breast cancer by targeting p27 (Miller et al., 2008). As discussed earlier, BCSCs express a high level of ALDH1. As a detoxifying enzyme, ALDH1 and 3 in cells metabolizes the anti-cancer agents such as cyclophosphamide and its analogs like ifosfamide, mafosfamide, and 4-hydroperoxy cyclophosphamide (Parajuli et al., 2014). All these reports indicate that targeting CSC-mediated drug resistance might be useful to control tumor relapse and increase disease-free survival.

6.4. Role of breast cancer stem cells in tumor recurrence

Tumor initiation and progression are two important processes in cancer development. Tumor initiation requires two basic phenomena known as self-renewal and differentiation. CSCs are shown to maintain their own population throughout the life by a process called self-renewal and also produce phenotypically different neoplastic cells which contribute to tumor bulk by the process of differentiation. Self-renewal is a type of cell division where one or both of the descendant cells remain to be stem cells which maintain its pool. Self-renewal and differentiation of CSCs are tightly controlled processes and can be

Table 1
Role of miRNAs in regulating CSC-mediated breast cancer progression and drug resistance.

miRNA	Function	Mode of action
miR-24	1 Stemness maintenance 2 Increases drug resistance	1 Downregulation of FIH1 (Roscigno et al., 2017) 2 Downregulation of BimL (Roscigno et al., 2017)
miR-221/222	1 Stimulates BCSC phenotype. 2 Increases drug resistance	1 Activation of Akt/NF-κB mediated COX-2 expression (Li et al., 2016) 2 Targeting p27 (Miller et al., 2008)
Let-7c	1 Decreases the self-renewal of CSCs 2 Reverses drug resistance	1 Downregulates the estrogen receptor expression by binding to 3'UTR region of ERα gene and inhibits the activation of Wnt signaling by ERα (Sun et al., 2016) 2 Enhances the anti-tumor activity of tamoxifen by regulating the self-renewal property of CSCs (Sun et al., 2018)
Mir-208a	Increases the self-renewal of BCSCs.	Inhibits the expression of Let-7a (Sun et al., 2015)
miR-10b	Increases the self-renewal of CSCs	Down regulating the expression of PTEN (Bahena-Ocampo et al., 2016)
miR-27a	Enhances endothelial properties in BCSCs.	Expression induced by VEGF (Tang et al., 2014)
miR-142	Increases stemness in BCSCs	Activates the Wnt signaling via decreasing the APC level (Isobe et al., 2014)
miR-374a	Increases metastasis	Activation of Wnt signaling by inhibiting PTEN and WIF (Cai et al., 2013)
miR-600	Silencing causes increased stem cell phenotype.	Silencing causes activation of the non-canonical Wnt signaling pathway (El Helou et al., 2017)
miR-340	Decreases migration, invasion, and metastasis	Downregulation of Wnt target genes (Mohammadi-Yeganeh et al., 2016)
miR-489	Reverse Drug resistance	Reverses the drug resistance in BCSCs by targeting a key apoptotic protein XIAP (Wang et al., 2017)

regulated by both intrinsic and extrinsic signals. Mutations in the genome are type of the intrinsic factors that induce uncontrolled activation of stem cell-self-renewal pathways such as Wnt, Notch and Hedgehog that results in the conversion of normal stem cells into neoplastic stem cells (Fig. 3; Economopoulou et al., 2012). Self-renewal of CSCs is also regulated by extrinsic factors such as the interaction of CSCs with stromal cells such as fibroblasts, immune cells and endothelial cells and, stroma-derived growth factors and chemokines (Albini et al., 2015).

Several reports suggest that Wnt/β-catenin signaling pathway is important for stem cell self-renewal. It has shown that inhibition of Wnt pathway by pyrvinium pamoate reduces the self-renewal and dissemination of BCSCs (Xu et al., 2016a). Let-7c is a miRNA and it acts as a tumor suppressor by inhibiting the CSC self-renewal in ER + ve breast cancer through the downregulation of estrogen receptor (ER) expression thereby abrogating the activation of Wnt signaling (Sun et al., 2016). Let-7 was also shown to enhance the anti-tumor activity of tamoxifen by regulating the self-renewal property of CSCs (Sun et al., 2018). A recent study has also determined that JAK/STAT3 pathway-controlled fatty acid β-oxidation is crucial for self-renewal of BCSCs (Wang et al., 2018). Fascin, an actin-binding protein is important for maintaining CSC self-renewal by activating Notch signaling (Barnawi et al., 2016). Inhibition of Mammalian target of rapamycin (mTOR) phosphorylation by rapamycin induces the sensitivity of BCSCs towards the radiation therapy (Lai et al., 2016).

It has been well established that microRNAs have a key role in the regulation of BCSC self-renewal. Overexpression of MIR-208a increases the self-renewal of BCSCs by inhibiting the expression of Let-7a (Sun et al., 2015). PI3K/Akt signaling pathway is also imperative for the regulation of CSC self-renewal. Moreover, PI3k/Akt signaling is known to be negatively regulated by PTEN. Overexpression of miR-10b increases the self-renewal of CSCs by targeting the expression of PTEN (Bahena-Ocampo et al., 2016). In contrast, other miRNAs like miR-100 and miR-200C were shown to inhibit BCSC self-renewal (Deng et al., 2014; Feng et al., 2015).

7. Therapeutic targeting breast cancer stem cells for the management of breast cancer

CSCs have been implicated in tumor angiogenesis, metastasis, and drug resistance thus therapeutically targeting CSCs might beneficial for the treatment of breast cancer patients. BCSCs can be targeted by affecting different functional and molecular aspects of the BCSCs such as BCSC markers, signaling pathways responsible for self-renewal, tumor-stroma interaction and CSC-driven drug resistance pathways.

7.1. Therapeutic targeting of breast cancer stem cell markers

The most common CSC markers used for the isolation of BCSCs are: $CD44^+/CD24^-$ (Shao et al., 2016), CD133 (Brugnoli et al., 2017), GD2 (De Giorgi et al., 2011) and ALDH1 (Ginestier et al., 2007). These markers have both phenotypic and functional significance in the maintenance of BCSCs. Thus, therapeutically targeting these markers can be an important approach to eradicate CSCs. CD44 is a cell surface receptor for hyaluronic acid (HA). The interaction between CD44 and HA can be used to target CD44. Paclitaxel and rapamycin are well-known anti-cancer drugs. It has been reported that paclitaxel or rapamycin-carrying nano-carriers coated with HA have been shown to exhibit better efficacy than those without HA-coating (Agrawal et al., 2018; Yang et al., 2013; Zhao et al., 2014b). Knocking down CD44 has also shown to make BCSCs more susceptible to the anti-tumor drug, doxorubicin (Van Phuc et al., 2011). Lentivirus-mediated knockdown of CD44 also opens up a new avenue of therapy known as differentiation therapy. CD44 is being an important molecule responsible for maintenance of stemness in BCSCs, knocking down CD44 induces differentiation of the CSCs and thus, leads to eventually enhanced susceptibility to various therapeutic regimens (Pham et al., 2011).

CD133 is a cell surface glycoprotein, usually identified by a commercially available antibody which binds nonglycosylated epitopes. However, these nonglycosylated epitopes are lost during differentiation (Kemper et al., 2010). Thus, the main struggle was to find a region in CD133 which can be used as epitope under every condition. Swaminathan et al. (2010) have identified highly immunogenic amino acids from CD133 protein to develop an antibody against it. The gene encoding scFv portion of anti CD133 was fused to gene encoding deimmunized PE_{38}KDEL to generate an immunotoxin that can directly target CD133 molecule and thus, can target BCSCs (Ohlfest et al., 2013). AC-133 is an established commercially available monoclonal anti-CD133 antibody. Saponin, a well-known toxin and is highly used for the generation of immunotoxins. Conjugation of AC-133 with saporin (AC133-saporin) causes arrest in cell proliferation in CD133 cells. Receptor-mediated endocytosis is essential for target-specific delivery of drugs of immunotoxins into tumor cells. However, immunotoxin therapy is less successful due to low rate of drug penetration through endocytic vesicles and lysosomal degradation of therapeutic regimen. New innovative light-based technology termed as photochemical internalization (PCI) has been used to circumvent this problem. PCI aids in the release of macromolecules from endosomes based on photosensitizer-dependent rupture. Usage of Photo Chemical Internalization (PCI) leads to endosomal escape of AC133-saporin in CD133+ BCSCs leading to proliferation arrest and cell death (Bostad et al., 2015).

GD2, a glycosphingolipid is another cell surface marker of BCSCs. It

has been revealed that breast cancer cell line or clinical sample-derived GD2+ cells show stemness properties. GD3 synthase, the rate-limiting enzyme for biosynthesis of GD2 can be used as a therapeutic target for the management of BCSC phenotype. Targeting GD3 synthase using either by shRNA or small molecule triptolide reduces the CSC enrichment and abrogates primary tumor formation (Battula et al., 2012). Several studies are undergoing to generate genetically engineered anti-GD2 antibodies and evaluate the translational efficiency of same (Ahmed and Cheung, 2014). Aldehyde dehydrogenase 1 (ALDH1), unlike other conventional surface markers, is an enzyme. The activity of ALDH1 is positively correlated to stemness in BCSCs. ALDH1 is not just a phenotypic marker but also has a functional role in maintaining stemness. Thus targeting ALDH1 can be a useful therapeutic intervention to eradicate BCSCs. Withaferin A has been reported to target ALDH1 and subsequently causing BCSCs to lose its stemness (Kim and Singh, 2014). Another novel method by which ALDH1 + ve BCSCs can be targeted and eliminated is photothermal therapy-mediated highly crystallized iron oxide nanoparticles (Paholak et al., 2016).

7.2. Targeting self-renewal pathways of breast cancer stem cells

Self-renewal is one of the properties that separate CSCs from other types of cells in a heterogeneous tumor population. Self-renewal attributes of the BCSCs are regulated by certain signaling pathways such as Notch (Kang et al., 2015), Wnt/β-catenin (Khramtsov et al., 2010), Hedgehog (Tanaka et al., 2009), Hippo pathway (Maugeri-Saccà and De Maria, 2016), NF-κB (Kendellen et al., 2014) and RTK (Butti et al., 2018). Gamma-secretase is an important part of the notch signaling and essential for the cleavage of ICD domain. Targeting γ secretase with gamma-secretase inhibitors (GSI) like MK-0752 and PF-03084014 in BCSCs makes them more responsive to conventional chemotherapeutic agents like docetaxel (Schott et al., 2013; Zhang et al., 2013). Capsaicin, a chili pepper-derived compound induces apoptosis in BCSCs by inhibiting translocation of NICD into the nucleus (Shim and Song, 2015). A recent study has revealed the role of Vitamin D compounds, 1α,25(OH)2D3 and Gemini analog of vitamin D, BXL0124 in regulating BCSC differentiation and subsequent reduction of BCSC population by inhibiting Notch pathway. The vitamin D compounds specifically inhibit the expression of Notch signaling components such as Notch1, Notch2, Notch3, JAG1 and JAG2 (Shan et al., 2017). Targeting Wnt pathway give another lucrative avenue for controlling BCSC population. LGK974 is a drug that has been reported to target porcupine, a Wnt pathway associated acyltransferase (Liu et al., 2013c). The drug is currently in phase I clinical trial. Tankyrase, a member of the PARP family increases the degradation of axin, a component of Wnt pathway. Tankyrase inhibitors, XAV939 and IWR-1 inhibit both the isoforms of tankyrase and thus regulate degradation of axin (Krishnamurthy and Kurzrock, 2018). Celecoxib, a common non-steroidal, anti-inflammatory drug is reported to eradicate BCSCs by inhibiting the Wnt/β-catenin pathway (Huang et al., 2017). Hedgehog (HH) pathway plays a very important role in maintaining and regulating stemness in BCSCs. Thus targeting HH pathway could be one of therapeutic approaches for the removal of CSC population from mammary tumors. GANT61, a non-canonical inhibitor of HH has been reported to reduce the CSC population in TNBCs (Koike et al., 2017). *Trametesro biniophila* murr (Huaier extract) is a Traditional Chinese Medicine that has shown to inhibit stem-like properties and subsequently reduce the BCSC population (Wang et al., 2014a, 2014b). Genistein, a predominant isoflavone found in soy products, has also been used to reduce BCSC population (Fan et al., 2013). The Hippo is a more recently discovered player to have a role in the maintenance of stemness in BCSCs. Therefore, this pathway is under investigation to find out potential targets and drugs. Porphyrin compounds were identified as the most potent agents in inhibiting TEAD-YAP among various screened compounds and are presently under clinical trial (Maugeri-Saccà et al., 2015). Activation of NFκB pathway is customary in the maintenance of BCSCs. Thus, various anti-inflammatory molecules are widely explored as anti-neoplastic and chemopreventive agents. Aspirin, in particular, has shown the significant anti-BCSC property. However, gastrointestinal toxicity has put a limitation on its use. However, aspirin ester products seem to be providing the same desired result without any toxic effects (Kastrati et al., 2015). For hormone responsive form of breast cancer, various estrogen modulators such as tamoxifen or raloxifene are used as adjuvant therapy (Mirkin and Picker, 2015). Butti et al. (2018) have reviewed that EGFR can also be a lucrative target for anti-BCSC therapy as EGFR is frequently mutated or overexpressed in various types of breast cancer. The various anti-EGFR therapeutic agents are available including small molecular inhibitors and the monoclonal antibodies.

7.3. Targeting tumor-stroma interaction to obliterate cancer stem cell phenotype

Stromal cells exhibit a context-dependant cross-talk with the tumor cells through the secretion of an array of molecules such as leptin, SDF-1, IL-6, CXCL2, OPN, etc. Leptin is involved in a cross-talk between adipocytes and fibroblasts with cancer cells in breast tumor microenvironment to induce EMT and expression of stemness-related genes (Andò et al., 2014). Initial attempts were made to block free soluble leptin by using recombinant leptin binding domains (Niv-Spector et al., 2005). However, researchers have found various limitations targeting leptin. Then, several studies were tried to block the leptin receptors using synthetic receptor binding fragments (Otvos et al., 2008). Other modes of therapeutically targeting leptin are synthetic peptide based. The first leptin antagonist to be designed was a mutant variant of human leptin harbouring the Arg128Gln mutation (Raver et al., 2002). Moreover, leptin activity could inhibited by short peptides that is identical to 70–95 amino acid region of human leptin (Gonzalez et al., 2009). OPN, a member of the SIBLING family plays a major role in regulating and maintaining stemness in breast cancer (Pio et al., 2017). It has been reported that Tiam1 expression in breast CAFs induces BCSC through fibroblast OPN secretion, thus rendering it a potential therapeutic target in the field of BCSCs (Xu et al., 2016b). Blocking the expression of OPN/it's signaling with aptamers or by blocking the OPN receptor, αvβ3 with LM609 has reported to reduce BCSC population (Ahmed et al., 2011).

7.4. Targeting cancer stem cell-mediated drug-resistance

Although breast cancer patients display initial response to various treatments, most of the patients develop resistance to therapeutic regimen. The Aurora-A kinase together with Smad5 bestows chemoresistance in BCSCs through a noncanonical pathway. However, this drug resistance induced by Aurora-A/smad5 axis is abrogated when BCSCs treated with alisertib, a selective Aurora-A kinase inhibitor (Opyrchal et al., 2017). BRCA1 haploinsufficiency-driven RANKL gene overexpression induces chemoresistance in BCSCs. The biguanide metformin has been reported to re-sensitize the BCSCs to RANKL antibody, denosumab leading to the reversal of drug resistance followed by a reduction in BCSC population (Cuyas et al., 2017).

Trastuzumab has shown significant benefits clinically in the treatment of Her2 + ve breast cancer. However, due to BCSC-mediated drug resistance through p95Her2 and other Her family members, the response becomes limited. A potent anthelmintic agent, Flubendazole (FLU) is reported to reverse this resistance thereby increasing the potency of trastuzumab. FLU treatment induces apoptosis by significantly downregulating truncated p95Her2, p-Her2, p-Her3 and p-Akt levels, and suppressing hetero-dimerization of Her2/Her3. FLU also targets $CD44^+/CD24^-$ phenotype and ALDH1 expression (Kim et al., 2018). One of the mechanisms by which BCSCs induces drug resistance is by dysregulation of Bcl2 family of proteins. Sabutoclax, a Bcl2 family protein antagonist has been reported to show anti-cancer activity both *in vitro* as well *in vivo* cancer models. Sabutoclax in combination with

other chemotherapeutic agents has presented a strong synergistic anti-proliferative effect. Mechanistically, blocking BCL-2, BCL-xL, MCL-1 and BFL-1 by sabutoclax promotes the activation of caspase-3/7 and caspase-9 and modulates the expression of Bax, Bim, PUMA, and survivin that subsequently destroys BCSC population (Hu et al., 2018b).

BCSCs express low levels of miR-489 and are known to show resistant to the treatment of 5-fluorouracil. However, when miR-489 was overexpressed in breast cancer cells, the BCSCs show marked increase in their response to 5-fluorouracil. miR-489 reverses the drug resistance in BCSCs by targeting a key apoptotic protein, XIAP (Wang et al., 2017). The secoiridoid decarboxymethyl oleuropein aglycone (DOA), a phenolic compound obtained from extra virgin olive oil can selectively targets BCSCs. DOA when administered in combination with mTOR inhibitor, rapamycin or the DNA methyltransferase (DNMT) inhibitor, 5-azacytidine, synergetic anti-tumor effects were observed (Corominas-Faja et al., 2018). ABCG2 is intrinsically related to drug resistance in CSCs. A novel method involving Ultrasound (US) and microbubbles (MBs)-mediated reversal of doxorubicin resistance in BCSCs has been reported. The mechanistic study revealed that US-MBs target ABCG2 and thus, reverse the drug resistance in BCSCs (Guo et al., 2017). The various drugs and techniques to target BCSCs described here have been compiled in Table 2.

7.5. Cancer stem cell targets as adjuvant therapy

Neoadjuvant therapy or preoperative therapy is widely used in the treatment of locally advanced breast cancer and it is given before the surgery. Chemo and radiation therapies are widely used as a part of neoadjuvant therapy. It allows the tumor to shrink and make it easy to be removed by surgery. Even after traditional cancer treatments and surgery, tumor relapse is a common problem that occurs due to the residual cancer cells. Treatment which is given in addition to the primary treatment or surgery to enhance the efficiency of primary treatment and to prevent the chances of relapse is called as adjuvant therapy. It is well known that CSCs play a key role in tumor relapse as they are resistant to chemo and radiation therapies which are given in primary cancer treatment. Moreover, because of their property of circulating in the bloodstream they might present in the patient body even after breast cancer surgery. Targeting CSCs in adjuvant therapy can be used as a strategy to prevent the relapse of breast cancer.

Recently lysine-specific demethylase 1 (LSD1) shown to have a role in EMT, cancer stemness and therapeutic resistance in breast cancer. It was also observed that CTCs express higher levels of LSD1 in metastatic breast cancer patients. Targeting LSD1 by pharmacological inhibitors reduces the stem cell-like signatures in patient-derived CTCs. Hence targeting LSD1 was proposed to be used as promising adjuvant therapy to prevent the tumor relapse of breast cancer (Boulding et al., 2018). It was identified that mammospheres which contain an enriched number of CSCs show enhanced expression of surface antigen xCT in HER2 + ve breast tumor cells such as TUBO cells. Mice implanted with cells derived from tumorspheres showed reduced metastasis upon immunotargeting of xCT by DNA vaccination, suggesting the use of xCT inhibitors in adjuvant therapy may help in preventing tumor relapse (Lanzardo et al., 2016). Growing pieces of evidence suggest that metformin can also act as a potential adjuvant agent as it showed to inhibit tumor aggressiveness and promote CSC depletion (Barbieri et al., 2015; Zhang and Guo, 2016). It is also well known that NF-κB is an important signaling pathway that regulates CSC phenotype. Use of NF-κB inhibitor such as IMD-0354 reduces the CSC phenotype and expression of ABC transporters and induces the apoptosis of non-stem cells (Gomez-

Table 2
Targeting CSCs using different modalities.

DRUG/TECHNIQUE	TARGET	MODE OF ACTION
HA tagged nano-carriers	CD44	Nano-carriers carry known anticancer drugs. HA tagging increases efficiency of drug delivery based on CD44-HA interaction (Agarwal et al., 2018; Yang et al., 2013; Zhao et al., 2014b)
CD44 siRNA	CD44	Makes BCSCs more susceptible to doxorubicin. (Van Phuc et al., 2011)
scFv-PE_{38}KDEL	CD133	Induces cytotoxicity (Ohlfest et al., 2013)
AC133-saporin	CD133	Arrests cell proliferation (Bostad et al., 2015)
Withaferin A	ALDH1	Downregulation of Sox2, Oct4, nanog and Bmi-1 (Kim and Singh, 2014)
Photothermal therapy (PTT)	ALDH1	Inducing localized hyperthermia (Paholak et al., 2016)
MK-0752 (γ-secretase inhibitor)	Notch signaling	Makes BCSCs more susceptible to docetaxel (Schott et al., 2013)
PF-03084014 (γ-secretase inhibitor)	Notch signaling	Makes BCSCs susceptible to known chemotherapeutic agents (Zhang et al., 2013)
Capsaicin	Notch signaling	Prevents translocation of Notch intracellular membrane domain (NICD) into the nucleus. (Shim and Song, 2015)
1α,25(OH)2D3 (Vitamin D derived compound)	Notch signaling	Inhibits Notch1, Notch2, Notch3, JAG1, and JAG2. (Shan et al., 2017)
LGK974	Wnt signaling	Targets porcupine a Wnt pathway associated acyltransferase (Liu et al., 2013c)
XAV939	Wnt signaling	Tankyrase inhibitor (Krishnamurthy and Kurzrock, 2018)
Celecoxib	Wnt signaling	Inhibits the synthesis of prostaglandin E2 and down-regulating the Wnt pathway activity (Huang et al. 2017)
GANT61	Hedgehog pathway	Downregulation effector molecules in the Hh pathway: glioma-associated oncogene (GLI) 1 and GLI2 (Koike et al., 2017)
Huaier extract *(Trametesro biniophila murr)*	Hedgehog pathway	Inhibits stemness genes (Wang et al., 2014a)
Genistein	Hedgehog pathway	Downregulation of HH-GLI1 pathway (Fan et al., 2013)
Arg128Gln Leptin	Tumor stroma interaction	Inhibits leptin from binding to its cell surface receptor (Raver et al., 2002)
Short anti leptin peptide (amino acids 70-95)	Tumor stroma interaction	Inhibits leptin from binding to its cell surface receptor (Gonzalez et al., 2009)
Osteopontin aptamers	Tumor stroma interaction	Blocks the activity of secreted osteopontin (Ahmed et al., 2011)
LM609	Tumor stroma interaction	Blocks Osteopontin receptor, αVβ3. (Ahmed et al., 2011)
Alisertib	Drug resistance	Inhibits Aurora-A kinase (Opyrchal et al., 2017)
Metformin	Drug resistance	Sensitizes BCSCs to RANKL antibody, denosumab (Cuyas et al., 2017)
Flubendazole	Drug Resistance	Sensitizes BCSCs to trastuzumab (Kim et al., 2018)
Sabutoclax in combianation with chemotherapeutics	Drug resistance	Sensitizes BCSCs to chemotherapeutic agents by inhibiting BCL-2, MCL-1, BCL-xL and BFL-1 (Hu et al., 2018b)
Secoiridoid decarboxymethyl oleuropein aglycone (DOA)	Drug Resistance	Synergistic effects with mTOR inhibitors and DNA methyltransferase inhibitors (Corominas-Faja et al., 2018)
Ultrasound (US) and microbubbles (MBs)	Drug Resistance	Targets ABCG2 and inhibits its function (Guo et al., 2017)

Cabrero et al., 2013). These results indicate that targeting the pathways which are important for CSC phenotype in adjuvant therapy may help in reduction of tumor relapse and better outcome of cancer treatment.

8. Conclusion and future prospective

Several therapeutic interventions have been devised for the management of breast cancer based on its subtypes, molecular signature and mutational status. Nonetheless, intrinsic or acquired resistance to treatment modalities poses major confronts for anticancer therapy and facilitates the tumor recurrence. Decades of research on failure of chemotherapies have identified minute population of drug resistant cells that reside in solid tumors. This population shows stem-like properties and high tumorigenic potentials. Lack of information on generation and existence of drug resistant clones limits them as drug targets. Single cell analyses of tumors enable the identification of tumor heterogeneity and drug resistant clones. Tumor-stroma exchanges foster the development and enrichment of CSCs. The knowledge to target this stroma-dependant evolution of CSCs is still inadequate. CSC population acquired the principle pathways from normal stem cells to maintain their self-renewal and differentiation capabilities. Several drugs have been tested for diminishing the CSC for the treatment of cancer. However, the targeting these pathways poses formidable challenges as therapeutic regimens might destroy the normal stem cells in the human body. Hence, identification of CSC-specific signaling networks are essential for the betterment of anti-CSC cancer therapy. BCSCs display immense potential to migrate various parts of the body through the blood circulation as there are resistance to anoikis. After homing to distant organs, they remain dormant as a residual disease to resist therapeutic effects and inhibitory cues derived from target organ microenvironment. Hence, these cells are responsible for cancer relapse after protracted period of dormancy. Indeed, the recurrent form of cancer is more aggressive than primary tumors as these are developed from the drug resistance clones. Developing theranostic interventions to detect and target the residual disease at dormant stage might be useful for the management of recurrent form of breast cancer.

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