

Targeted Therapy Against BCSCs

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ABSTRACT

The population of women suffered from breast cancer accounts for the largest proportion in female cancer patients all over the world. Although numerous conventional therapeutic strategies have been developed to treat this disease, therapies for metastatic breast cancer remain inadequate. Breast cancer stem cells (**BCSCs**) are believed contributing as a source of the problem. BCSCs are a long-lived subpopulation of cancer cells involved in self-renewal, tumorigenesis, aggressiveness, radioresistance and chemoresistance. In this review, we will focus on the recent studies regarding BCSC-based targets, relevant signaling pathways, proposed mechanisms causing chemo- and radioresistance and suggested targeted therapy against BCSCs.

Keywords: BCSCs; Biomarkers; Microenvironment; MicroRNA; Resistance mechanisms; Signaling pathways

Abbreviations: ABC: ATP-Binding Cassette; ALDH: Aldehyde Dehydrogenase; BCSC: Breast Cancer Stem Cell; CSC: Cancer Stem Cell; DSB: Double-Strand Breaks; EMT: Epithelial-Mesenchymal Transition; ER: Oestrogen Receptor; GSI: γ -Secretase Inhibitor; HER2: Human Epidermal Growth Factor Receptor 2; HIFs: Hypoxia Inducible factors; HH: Hedgehog; MDR: Multidrug Resistance; miRNA: MicroRNA; MSC: Mesenchymal Stem Cells; MSC: Mesenchymalstem Cells; NF- κ B: Nuclear Factor Kappa B; RA: Retinoic Acid; ROS: Reactive Oxygen Species; TNBC: Triple-Negative Breast

INTRODUCTION

The population of women who suffered from breast cancer accounts for the largest proportion in female cancer patients all over the world. It is responsible for the greatest number of cancer deaths among women after lung cancer and approximately 90% of mortality is due to metastasis [1,2]. Conventional therapies, such as surgery, radiotherapy, chemotherapy, hormone therapy and bone-directed therapy, have been applied to treat this disease [3]. Drugs approved by FDA (U. S. Food and Drug Administration) to treat breast cancer have been listed on the web page (<https://www.cancer.gov/about-cancer/treatment/drugs/breast>) published by NCI (National cancer institute) of NIH (National institutes of health). However, it is well known that the chemotherapy and radiotherapy have tremendously side effects [4]. Worsely, the cancer cells evolve into chemo- and radioresistance [5].

These problems can be attributed to the breast cancer stem cells (**BCSCs**), a sub-group of cancer cells with stem cell properties [6]. Bonnet et al. first time verified that human acute myeloid leukemia originated from acute myeloid leukemia stem cells. BCSCs were first identified and isolated by Al-Hajj et al. in 2003. They demonstrated that as few as 100 cells with CD44+CD24-/lowLin- phenotype injected to the non-obese diabetic/severe combined immunodeficient (**NOS/SCID**) mice were able to induce tumorigenesis, while 100 times quantities other alternate phenotypes cell failed to generate tumor. It indicated that breast cancer suffered from BCSCs [7].

Currently, accumulated evidence has shown that BCSCs have the capability of proliferation, differentiation, tumorigenesis and self-renewal, leading to chemo- and radioresistance, metastasis and tumor relapse [8]. Therefore, the therapies target BCSCs may be a more efficiency strategy in the breast cancer treatment [9].

BCSC-BASED TARGETS

Regarding identification and determination of molecular targets of BCSCs, they are complicated by different dimensions: (1) cross talking with relevant factors in the vast network among Notch, Hedgehog, TGF- β and VEGF/VEGFR-2 signaling pathways, inflammatory cytokines, oncogenic kinases and transcription factors; (2) combination with other factor(s), such as PIWIL2 plays roles with piR-932; (3) negative or low expression, such as CD24; (4) induction by epithelial mesenchymal transition (**EMT**) from different situations; (5) multifunctional property, such as ROR γ positively regulates DNA-repair, but negatively regulates the oncogenic TGF- β /EMT and MaSC pathways; (6) uncertainty counterpart(s) in pre-existing metastatic niches. So, just currently interested targets including biomarkers, key mediators of relevant signaling pathway and immunoreactions are summarized below.

BCSC Biomarkers

BCSCs have been reported to contribute to the tumor cell heterogeneity [10]. A number of

biomarkers have been reported to identify BCSCs (Table1). The biomarkers of BCSCs commonly are transmembrane protein, such as CD44, located in the surface of BCSCs, which could serve as a hub to connect extracellular microenvironment and cytoplasm [11]. Moreover, expression of certain biomarkers have been reported to be linked with the initiation, invasion, metastasis, and therapy-resistance of breast cancer, as well as poor prognosis [12-14]. The biomarkers, such as CD133 and aldehyde dehydrogenase 1 (ALDH1), could be a new target for overcome drug resistance in the breast cancer treatment [15,16].

Table1: BCSCs biomarkers were utilized for the identification of phenotype.

BCSCs biomarkers	phenotypes
CD44	CD44 ⁺ /CD24 ^{-low} Lin ⁻
CD24	
ALDH	ALDH1 ⁺ [17]
CD133	CD44 ⁺ /CD24 ⁻ CD133[18]
CD49	CD49f ⁺ /CD61 ⁺ [19]
CD61	
CD90	CD90 ⁺ /CD14 ⁻ [20]
CD14	
PROCR	PROCR ⁺ /ESA ⁺ [21]
ESA	
CD29	PR / ER / HER2 [22]
Side population	Side population [23]

Key Elements of Relevant Signaling Pathways

It is well known that signaling pathways play an important role in the regulation of BCSCs properties, which increased chemo- and radioresistance, promoting tumor growth, metastasis, and invasion [24]. Elements along the signaling pathways can serve as modulators involved in the important programs of BCSCs, such as sex hormones and their receptors [25], particular genes and their expression products related to development of cancer [26], apoptosis [27] and angiogenesis [28]. The molecules in these programs have been reported to be utilized as therapy targets for BCSCs-based breast cancer treatment (Table2).

Table 2: Signaling pathways based targets in BCSCs.

Signaling pathways	Therapy targets
Sex hormone receptors	ER, PR, AR [29]
Gene expression modulators	BRCA1, BRCA2, p15, p16, PTEN [30], Ha-Ras, c-Myc [26], ErbB2 [31]
Apoptosis modulators	Surviving [32], P53 [25], Fas, Bcl-2 [33], galectin-3 [34], Bcl-xL [35]
Angiogenesis modulators	BET [36], SET7 [37], DEK [38], TBX3 [39], ROBO4 [40]

Immunological Mediators

The relationship between BCSCs and immune system has been widely investigated. BCSCs are able to induce the immune escape response to prevent immune cells, such as NK cell, mediated killing, leading to tumor growth and metastasis [41]. It has been continually attracted scientists to identify new targets for the BCSCs-based immunotherapy. Currently interested targets related to immunotherapy are summarized in Table3.

Table 3: Immunotherapy targets for BCSC-based treatment.

Mechanisms of BCSC-based treatment	Therapy targets
Immunological mediators	MICA, MICB [41], CCR7, OX2,CXCR1,CXCR2 [42], HSP [43], CCL5, INF- γ [44] TNF α , TGF β [45]

RESISTANCE MECHANISMS INVOLVING BCSC

The BCSCs have been reported to play significant roles in chemo- and radioresistance. The resistance mechanisms are attributed to the ATP-binding cassette (**ABC**) transporters, ALDH activity, inhibition of apoptosis and DNA repair [24].

ABC Transporters

The ABC transporters were believed to balance the compounds and small molecules in and out of the cells. It has been reported to take participate in response to anticancer therapy [46]. The members of ABC transporter family, such as ABCB1, ABCG2 and ABCC2, were demonstrated to be important in multidrug resistance (**MDR**) and associated toxicity [47,48].

Up-regulation of ABCB1 would induce permeability glycoprotein (**P-gp**) overexpressing, which also has been verified to be responsible for many anticancer drugs efflux by ATP, leading to MDR phenotype in anticancer therapy [49,50]. Deeply, ABCB1 gene has been found to be in highly polymorphism, which was attributed to MDR [51]. Furthermore, ABCG2 is known as breast cancer resistance protein (**BCRP**), which also was thought as a BCSCs marker. The event that expression of ABCG2 in BCSCs has been thought to up-regulate resistance mechanisms and other cancer stem cell properties, such as differentiation, regeneration and invasion, leading to poor prognosis [52].

ALDH Activity

ALDH, known as a BCSCs marker, were reported to catalyse oxidation of cellular retinol to retinoic acid (**RA**), which was contributed to the cellular detoxification, differentiation, and drug resistance [53]. RA regulates its target genes by binding to RA receptor [54,55]. The latter binds to DNA to forming a complex that known as DNA-binding protein, which is able to react with the co-repressors to form a heteromultimer, thereby interacting with histone deacetylases (**HDACs**), leading to transcriptional repression [56,57]. By this retinol signaling, RA induces an event of cellular differentiation. For example, Ginestier et al. showed that the retinoid signaling based variation of ALDH activity induced BCSCs capabilities of self-regeneration and differentiation undergoing promotion or suppression [56].

ALDH1, mainly ALDH1A1, ALDH1A2 and ALDH1A3, has been revealed to be associated with oxidation of aldophosphamide and detoxification of some applied anticancer drugs, such as oxazaphosphorines [58,59]. ALDH1A1 is involved in oxidation response of acetaldehyde in cells, suppression of anti-apoptosis systems and generation of ROS, leading to CSCs phenotype, carcinogenesis [60]. Furthermore, ALDH1A1 and ALDH1A2 have been demonstrated to possess the ability of inactivation of 4-hydroperoxycyclophosphamide, which could drive CSCs radio- and chemoresistance [61,62].

Inhibition of Apoptosis

Suppression of apoptosis could enhance BCSCs chemoresistance property [63]. Therapeutic targeting of anti-apoptotic molecules in BCSCs has been developed [64]. Survivin, a member of inhibitor of apoptosis protein family (**IAPs**), is a considerable cluster protein that contributed to the anti-apoptosis and regulation of cell cycle [65]. A recent study showed that survivin acts as a marker for Quiescent-BCSCs [32]. Expression of survivin in breast cancer results in inhibition of apoptosis and cell death, leading to chemoresistance and invasion of tumors [66,67]. Furthermore, Tran et al. showed that the mechanism of survivin-resistance could be attributed to induction of VEGF expression [68].

In addition, down-regulation of reactive oxygen species (**ROS**) was verified to relate to inhibition of apoptosis in BCSCs [69]. ROS is a product of mitochondrial respiratory chain reaction, which plays an important role in promote DNA damage and cells apoptosis [70]. These BCSCs exhibit relatively radio- and chemoresistance by comparison with non-stem cells, including differentiated and proliferative cancer cell populations [24,69]. Importantly, studies have demonstrated that the apoptosis induced by ROS could be mediated by the MAPK pathways (c-Jun N-terminal kinases (**JNKs**) and p38) [71].

DNA Repair

Radiation treatment exerts its therapeutic effect by inducing complex DNA damage in cancer cells and eventually cell death, as well as by some chemotherapies [72]. Rather, DNA repair may play a critical role in a number of resistance mechanisms about inefficacy of currently available chemo- and radiotherapeutic agents. DNA damage, primarily DNA double-strand breaks (**DSB**) induced by radiotherapy, could be repaired via non-homologous end joining or homologous recombination, which could improve the efficacy of treatment [72]. It has been reported that DSB repair program could be conducted by MRN (**MRE11/RAD50/NBS1**) nuclease complex, known as able to promote DNA replication, leading to CSCs phenotype and tumorigenesis [73,74].

BCSCs are capable of enhanced repair of damaged DNA. Recently, a study suggested that DNA repair activity could be increased in the BCSCs because of the upregulation of DNA repair genes and the induction of the metabolic substrate fumarate, which inhibits JKD2B histone demethylase activity thereby contributing to enhance DNA damage resistance [75]. The non-stem cancer cells could be induced to death result from defective DNA repair mechanisms, whereas the CSCs survived [76].

Importantly, it has been reported that approximate 5% to 10% of all breast cancer were hereditary disorder, which was believed to correlative with DNA repair genes, such as BRCA1, BRCA2, PALB2, WRN, and MLH1, undergo mutations [77]. Among these genes, BRCA1 was reported that essential for DNA DSB damage repair by interacting with DNA-damage signaling protein such as the ATM-Chk2 and ATR-Chk1 pathway [78-80]. In addition, Phillips and colleagues have demonstrated that BCSCs have no change in phosphorylation of H2AX in irradiated, which means that BCSCs have the powerful DNA damage repair capability [81].

MICROENVIRONMENT AND BCSC

Recently, The concept of tumor microenvironment has already been proposed to explore the BCSCs properties, such as proliferation, differentiation and regeneration, in breast cancer [82,83]. Mutual regulation between normal cells and CSCs in tumor microenvironment, known as niche, consist of endothelial cells, mesenchymal stem cells (**MSC**), pericytes, immune and inflammatory cells, adipocytes, and fibroblasts [84,85]. There are three niche types has been reported for the maintenance and self-renewal of BCSCs. They are perivascular niche which nurtures and promotes BCSCs growth, invasive niche promote the tumor metastasis and hypoxic niche which induces BCSCs phenotype [86]. Recently, cells in niche have been found to influence the CSC state through paracrine signaling [87]. The signaling, such as CXCR4, CXCR7, MMP12, MMP13, IL-6 and TGF- β , were reported to be in relation to tumor metastasis [88]. Furthermore, growth factors, such as VEGFs, FGFs, HGFs were proposed about angiogenesis-relate for cancer cell growth. In addition, other cytokines, such as EGF, TNF- α and PDGFs, are able to induce CSCs undergoing EMT, serving as a critical step to the metastatic processes of BCSCs [85,89].

Borovski and colleagues showed that oxygen pressure, nutrients, and pH in the niches also could influence the differentiation of CSCs [90]. In the hypoxia condition, some proteins associated with maintenance of stemness in CSCs, such as octamer-binding transcription factor 4 (Oct4) and Notch1, have been expressed [91,92]. Moreover, hypoxia inducible factors (**HIFs**), heterodimers composed of HIF-1 α /HIF-2 α and HIF-1 β , could induce BCSCs phenotype [93]. Overexpressing of HIF-1 α can drive hypoxia response, which causes downstream target gene expression, leading to angiogenesis, migration, invasion, and metastasis [94]. For example, fibroblasts in breast cancer microenvironment could increase neoangiogenesis by secreted VEGF and FGF in the presence of HIF-1 α signaling, which could provide more oxygen and nutrients for the cancer cells excessive growth [95,96]. Expression of CAIX could be induced by the HIF-1 α , leading to cancer cell survival and invasion [97,98]. Additionally, HIF-2 α could increase octamer-binding transcription factor 4 (Oct4) expression and the transcriptional activity of c-Myc, enhancing cancer cells proliferation and tumor growth [91,99].

SIGNALING PATHWAYS TARGET BCSCs

BCSCs signaling pathways target therapeutic has become an important treatment strategy. Deregulation of these signaling pathways, such as Wnt/ β -catenin pathway, Nuclear factor kappa B (**NF- κ B**) pathway, Hedgehog (**HH**) pathway and Notch pathway in BCSCs contribute to development of breast cancer [100-102]. The key targets are summarized in Table 4.

Table 4: Transcriptional targets of signaling pathways.

Signaling pathways	Target genes
Wnt/ β -catenin	Bmi, AXin, SOX7, cyclin D1, c-Myc, Sam 68, Lef1, Tcf4, Cdh1
NF- κ B	IL-6, IL-8,CXCLs
Hedgehog	cyclin D1, Gli1, Smo, P63, Bmi1, Snai1, VEGF, CXCR4
Notch	HES1, HEY2, c-Myc, γ -secretase

Wnt/ β -Catenin Signaling Pathway

Wnt/ β -catenin pathway could be activated in several tumor types, including colorectal and breast cancer [103]. Abnormal activation of the Wnt/ β -catenin pathway is related to the maintenance of CSCs properties in BCSCs [104]. Wnt/ β -catenin pathway involves Wnt protein, frizzled protein, dishevelled, β -catenin, T cell transcription factor/lymphoid enhancer factor (**TCF/LEF**), APC complex contained APC protein, GSK-3 β and Axin. Aberrant activation of Wnt gene suppresses the process about β -catenin phosphorylated by GSK3 β /APC/Axin complex. It down-regulates the phosphorylate degradation of β -catenin, which could promote the increasing β -catenin nuclear translocation to combine with TCF/LEF and downstream genes overexpression [104-107]. Wnt/ β -catenin signaling pathway target genes including the inhibitor of proliferation, such as cyclin D1 and c-Myc, Wnt pathway components, such as Axin2, and stem cell genes, such as Bmi-1 and SOX7 [108-110]. Hence, down-regulating the Wnt/ β -catenin signaling may be critical for the suppression of BCSCs stemness and activity [111].

NF- κ B Signaling Pathway

As early as nineteenth century, the relationship between inflammation and cancer has been first proposed, which now has already been believed to be an essential part of carcinogenesis [112]. The inflammation response was believed to closely relate to the initiation and development of breast cancer [113]. The development of inflammation is regulated by inflammatory cytokines, which are involved in NF- κ B pathway and be linked to cancer initiation, metastasis and chemoresistance [114].

Members of NF- κ B family, including p50, p52, p65, c-Rel and RelB, usually are inactivated when they bind with I κ B family members, respectively, in cytoplasm of resting cell [115,116]. Activation and then nuclear transportation of NF- κ B due to I κ -B degradation induces the expression of inflammatory cytokines, such as IL-6 and IL-8 [117,118]. Studies revealed that IL-6 could promote tumor initiation under the particular condition, progression and tumor angiogenesis, and IL-8 was able to promote the self-renewal event of BCSCs by binding to the CXCR1, which was receptor of IL-8 [119,120].

Zhen and coworkers demonstrated that overexpression of interleukin-1 receptor-associated kinase 1 (**IRAK1**), upstream of NF- κ B pathway, promoted TNBC growth, maintain stemness and enhance chemoresistance by activating its downstream NF- κ B pathway, thereby increasing secretion of interferon signaling in inflammatory responses [121]. Therefore, NF- κ B signaling pathway play critical roles in cell inflammation and regulation of the BCSCs self-renewal [122].

HH Signaling Pathway

The HH signaling pathway consists transmembrane protein (Patched (**Ptc**) and smoothened (**Smo**)), multiple ligands (Sonic hedgehog (**Shh**), Indian hedgehog (**Ihh**), and Desert hedgehog (**Dhh**)) and multiple Gli transcription factors (**Gli1**, **Gli2**, and **Gli3**) [123]. As a HH receptor, Ptc binds to Hedgehog proteins (**Shh**, **Ihh**, **Dhh**) to activate Smo, leading to the activation of

downstream transcription regulators Gli, thereby inducing its target genes expression. Conversely, activity of Smo could be inhibited by Ptcin the absence of HH proteins [124].

The HH signaling pathway is enable to regulate BCSCs properties. It has been showed to promote chemoresistance through activating drug resistance mechanisms and anti-apoptosis mechanisms [125]. Regulation of the activity of key elements in HH pathway is crucial for the BCSCs self-renewal, proliferation and apoptosis. Fan and coworkers showed that Gnistein could decrease BCSCs through down-regulation of the HH-Gli1 Signaling Pathway [126]. Furthermore, Lu and coworkers demonstrated that down-regulation of HH signaling, via Smo and/or Gli1, by Salinomycin could inhibit the proliferation of BCSCs and induces apoptosis, which indicated that agent about modulation of HH signaling may be an attractive therapeutic target for breast cancer [127].

Notch Signaling Pathway

The Notch signaling pathway is involved in regulation of many vital cellular processes, including proliferation, apoptosis, initiation and progression of breast cancer, and self-renewal of BCSCs [128]. Thence, understanding the signal transduction mechanisms of Notch signaling pathway would be helpful for design an effective strategy of breast cancer treatment [129].

The Notch pathway consists of four Notch receptors (transmembrane proteins, such acNotch1 to Notch4), five DSL ligands (transmembrane proteins with large EGF-like repeats, such as Jagged1, Jagged2, Delta-like1 (**Dll1**), Dll3, and Dll4) and a complex with a DNA-bound transcription factor from the CSL family. The Notch receptor is subjected to proteolytic cleavage steps and release its intracellular domain (**Notch-ICD, NICD**) for nuclear translocation, which consequently activates the transcription program, leading to target genes, including HES1, HEY2 and c-Myc, be transcribed [130-132].

Drugs interfering BCSCs by targeting the Notch pathway has been investigated to improve clinical outcomes in breast cancer treatment. Jae et al. showed that Gemini vitamin D analog, BXLO124, inhibited Notch1 signaling pathway through rapidly inducing its downstream target gene HES1, resulting suppression of BCSCs [133]. Furthermore, γ -secretase inhibitor (**GSI**) has been used as an agent of BCSC-directed in breast cancer treatment. Studies showed that glucose functionalized nanoparticles carrying GSI could inhabit Notch pathway in BCSCs, which reduced the BCSC populations and inhibited breast tumor growth [134]. Increasing evidences showed that GSI treatment could weaken the BCSCs tumor-promoting ability by multiple methods, including reduction of BCSCs populations, reduction of mammosphere-forming efficiency (**MSFE**) and suppression of tumor recurrence [135].

MICRORNAS AND BCSCs

The regulation of genes based on transcription, such as degradation of target mRNA, can be executed by the microRNA (**miRNA**), which has no-coding ability and less than 25 nucleotides [136]. In gene regulator network, miRNAs have been demonstrated as a group of dominance

component. They bind to the special sides of the target mRNA, which usually means the 3'-untranslated region (**3'-UTR**) based on the rule of base complementation pairing, to trigger the degradation of the target mRNA and repression of the translation program, leading to suppression the expression of the target genes [137-140].

Evidences showed that miRNAs play vital roles in regulation of CSCs properties, which are considered to be potential therapeutic targets [141]. Shimono and coworkers demonstrated that the expression of miRNAs were different between human BCSCs and non-tumorigenic cancer cells. They affect the initiation and invasion of breast cancer by involving in the regulation of human BCSCs [142]. Previous studies have reported that miR-30c and let-7f were related to the BCSCs, their down-regulation were found not only to play significant roles in regulation of BCSCs properties, but also to suppress the mechanisms of chemoresistance, increasing the chemotherapeutic outcomes [136]. Therefore, reveal the mechanisms that how miRNAs regulate BCSCs may be a novel therapeutic target for breast cancer (Table 5).

Table 5: MicroRNAs regulate BCSCs.

Regulation of BCSCs	MicroRNAs
Up-regulation of BCSCs	miR- 9, 27, 106b, 125, 129, 135, 142, 140, 146, 150, 155, 199a, 205, 214, 221, 222, 424, 510, 661, 663, Let-7
Down-regulation of BCSCs	miR- 7, 8, 30, 31, 33b, ,34a, 34c, 96, 99a, 100, 128, 141, 182, 183, 200(a, b, c), 204, 335, 340, 429, 485

Emerging evidences demonstrated that miRNAs could be served either as a oncogenic factor or as a suppressor during the initiation and progression of breast cancer [111]. They also were able to regulate expression of the stemness-related genes through the signaling pathways, such as went signaling pathway, Notch1 signaling pathway and PTEN/Akt pathway [143-145]. For example, down-regulation of miR-204 increases expression of the 68 kDa Src-associated protein in mitosis (**Sam68**), an RNA-binding protein with signaling functions overexpressed in breast cancer, by activating Wnt/beta-catenin pathway, leading to tumorigenesis and promote BCSCs self-renewal potential [146,147]. Moreover, miR-221/222 serves as an enhancer to strengthen BCSCs properties, which was completed by down-regulation of PTEN and activating Akt signaling, suggesting that it could be a new target for the treatment of breast cancer [144]. In addition, emerging studies indicated that microRNAs enhance the self-renewal abilities of BCSCs through up-regulating the expression of Bmi gene, an important self-renewal factor [142].

Importantly, epithelial-mesenchymal transition (**EMT**) has been proposed to be a developmental process in which epithelial cells can be induced to become mesenchymal cells. In other words, those cells lose their epithelial characteristics, such as adherens junction, tight junction and desomosome, and gain regenerated potential, such as migratory and invasive properties, in EMT program [148]. In breast cancer, the aberrant activation of EMT program is involved in the metastasis and aggressive progression of tumor [149]. Recently, a numbers of studies showed that miRNA could also participate in regulation of EMT. For example, Hong and colleagues have reported that up-regulated expression of miR-25b increased the expression of

its downstream gene MAP2K7, which promoted EMT program, leading to poor prognosis and chemoresistance of triple-negative breast cancer (TNBC) [150].

BCSC-TARGETING AGENTS

BCSCs target chemotherapy was most prevalent strategy for the treatment of breast cancer. Agents have been made according to different mechanisms of BCSCs target therapy. As we know, ovarian steroid hormones are the risk factors for the breast cancer. The interaction between sex hormones and their receptors is able to influence BCSCs phenotype. In BCSCs target hormone therapy, agents target and block the receptors [29]. Under the similar principle, agents interference the BCSC-based signaling pathways for the therapeutic purpose. On the other hand, for BCSCs target immunotherapy, a certain number of immune-related cells, such as activated dendritic cells (DCs), Natural killer (NK) cells and cytokine induced killer cells are injected to directly capture and kill BCSCs [151,152]. Currently interested therapeutic targets in BCSCs (Figuer 1) could be utilized for the development of anti-cancer drugs. Some of such drugs have already been applied clinically and achieved well outcomes.

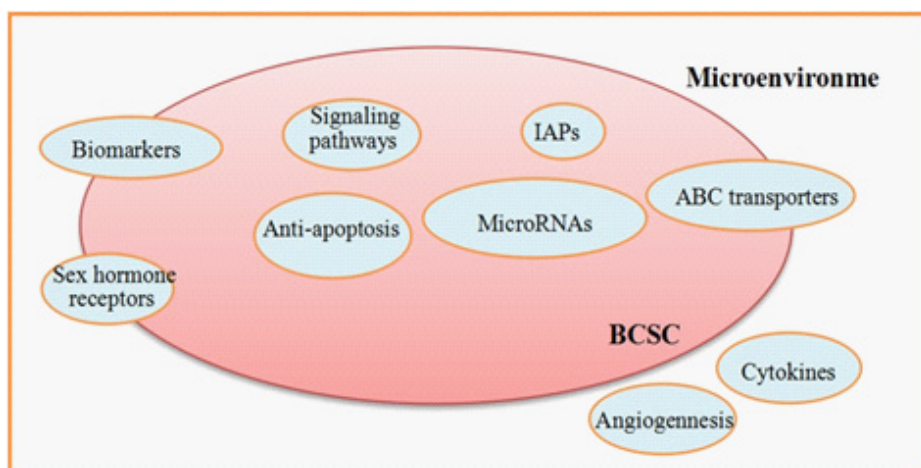


Figure 1: Putative therapeutic targets to suppress the activities of BCSCs.

Salinomycin

Salinomycin, a big macromolecule monocarboxylic polyether antibiotic, was first acquired from the fermentation of *Streptomyces albus*, which was well known to be used as an anti-parasitic drug, an anti-bacterial drug and a food additives [153-155]. It also has been known as an ionophore exhibits the strict selectivity for alkali ion and highly affinity for potassium, promoting potassium efflux, leading to suppression of oxidative phosphorylation program, cytotoxicity and apoptosis [155-157].

Salinomycin also has been reported to play an important role in the breast cancer treatment. Hyunsook et al. demonstrated that salinomycin suppressed breast cancer growth. They verified

that salinomycin downregulated the transcription factors, Nanog, Oct4 and SOX2, and BCSCs markers, ALDH1 and CD44, which induced the suppression of BCSCs and inhibition of tumor growth [158]. Furthermore, Niwa et al. have reported that salinomycin was able to induce the breast adenocarcinoma (**MCF-7**) cells apoptosis phenotype by suppression of BCL-2, BCL-XL and BIRC5 genes [159]. Expression of those genes would activate anti-apoptosis. It was consistent with the previous study that Salinomycin induced the activation of apoptosis pathway depending on P53, a protein could suppress the tumor growth and caspase activation [160]. In addition, salinomycin has been demonstrated that it exerts anticancer effects on BCSCs by involving in the down-regulation of hedgehog signaling pathway. Salinomycin could be served as a modulator of hedgehog pathway by markedly down-regulate the genes, including Smo and Gli, which would increase the suppression of BCSCs [127].

Lapatinib

On the population of breast cancer, there are approximately forty percent were HER2+, which overexpression of HER2, indicating poor prognosis [161]. Both HER and EGFR belong to the EGFR family, which are correlation with the growth factor signaling, blood vessel growth and CSCs proliferation tumor metastasis [162]. By heterodimerization, HER2 and EGFR often act synergically to promote breast cancer growth. HER2 potentiates EGFR signaling while the stimulation of EGFR induces activation of HER2 [163]. Therefore, the drug exhibits inhibition of both EGFR and HER2 ability may be the most effective agents [164].

Lapatinib is an oral formulation with the abilities of inhibition of both HRE2 and EGFR, which could be used to suppress HRE2+ breast cancer growth and metastasis [165]. Recent studies have showed that Lapatinib could increase the apoptosis and growth arrest of tumor cells, which were attributed to the inhibition of signaling pathways, such as MAPK pathway, Akt pathway and phosphatidylinositol 3-kinase (**PI3K**) pathway [166,167]. Lapatinib is allowed to combine with other drug, such as Capecitabine, to treat the HER2+ breast cancer. Cetin et al. demonstrated that by using Lapatinib plus capecitabine to treat the HER2+ breast cancer sufferers after chemotherapy would be effective and showed well toleration and mild toxicity [168].

Curcumin

Curcumin is a natural compound extracted from turmeric (**Curcuma longa**). It has been reported to involve in the resistance of biological oxidation, inflammation, infection and the most important that it also showed anticancer potential [169]. The mechanisms of its anticancer properties have been demonstrated relating to inhibition of signaling pathways, such as Wnt pathway, NF- κ B pathway [170,171]. In breast cancer, curcumin inhibits the growth and self-renewal of BCSCs by directly targeting BCSCs and inhibition of signaling pathways [172]. Moreover, Mukherjee et al. demonstrated that curcumin down-regulates expression of Slug, the important molecular involved in EMT program, up-regulates expression of E-cadherin, and increases beta-catenin in BCSCs, leading to suppression of EMT and migration of BCSCs [171].

In addition, Zhou et al. reported that curcumin was able to suppress BCSCs self-renewal by the inhibition of ABCG2, known as a member of ABC transporters family that contribute to the BCSCs self-renewal and therapy resistance [173]. Importantly, Anto et al. have proposed that curcumin could induce tumor cell apoptosis by down-regulating COX-2 gene, which has been demonstrated to suppress apoptosis program [174].

Aromatase Inhibitors

On the population of breast cancer, approximately three fourth are oestrogen receptor positive (**ER+**), which overexpression of ER, suggesting that ER can served as a therapeutic target for the treatment of breast cancer. Studies have been demonstrated that therapies target ER can be executed by aromatase inhibitors, which were able to suppress formulation of estradiol [29].

Anastrozole is a aromatase inhibitor that was usually used for the treatment of advanced breast cancer in post-menopausal women and was able to improve the survival rate [175]. Buzdar showed that anastrozole exhibit powerful capability of deplete the estrogen and fewer side effect than other hormonal therapies [176]. Moreover, anastrozole lead less weight gain compared to other agents, such as megestrol acetate [177].

Letrozole, another aromatase inhibitor, was able to inhibit the program of androgens to estrogens, thereby reducing the estrogens. It also can be used as an agent for the treatment of advanced breast cancer in post-menopausal women and generating better outcomes [178]. In addition, Letrozole could be used with other agents for inhibition of BCSCs. For example, Liu et al. demonstrated that letrozole plus everolimus administrate showed more effective in the inhibition of MCF-7 stem cells growth and tumorigenicity compared to either single agent administrate [179].

Trastuzumab

Trastuzumab is a monoclonal antibody (**mAb**) usually used as a HER2 inhibitor for the treatment of HER+ breast cancer. It has been demonstrated that trastuzumab was able to bind to the extracellular structure of HER2 [180]. By this manner, trastuzumab inhibits the releasing of extracellular structure of HER2, leading to the prediction of tumor response and advantage survival [181,182]. Moreover, recent studies have reported that combination trastuzumab with chemotherapy, such as paclitaxel, doxorubicin, and cyclophosphamide, in the adjuvant therapy could improve the clinic outcomes [183-185]. Importantly, Nagata et al. found that trastuzumab treatment could promote the activation of PTEN, which induced the suppression of breast cancer growth [186].

Oncolytic Virus

Oncolytic viruses are group of genetically modified viruses that preferentially infect and destroy cancer cells [187]. Recently, literatures have described that the DNA viruses, such as adovirus, herps simplex virus (**HSV**) and vaccinia virus, and RNA viruses, such as measles virus, reovirus and Newcastle disease virus (**NDV**), have been used as the oncolytic virus in tumor treatment

[188]. Marcato et al. have demonstrated that the oncolytic reovirus have capability of breast cancer regression. They found that the oncolytic reovirus could effectively infect and destroy the cells with the phenotypes of CD24–CD44+ and ALDH1+ BCSCs suggesting that oncolytic reovirus may be a novel therapeutic for the suppression of tumor by effective target BCSCs in breast cancer treatment [189]. Moreover, it has been proposed that the oncolytic virus could be equipped with extra molecules to directly target the cancer cells or other component in tumor niche for increasing the anti-cancer efficacy [188]. For example, Conner et al. employed oncolytic HSV1716 with overexpressing inhibitor of growth 4 (Ing4) to enhance its tumor destructive property. They found that the oncolytic HSV1716 arming with Ing4 showed more than 1000 times capability of BCSCs infection compared with the unmodified HSV1716 in breast cancer treatment [190]. In addition, oncolytic virus could be used with other drugs, such as paclitaxel, doxorubicin and bevacizumab, for improving their effectiveness in the treatment of breast cancer [191,192].

CONCLUSION

BCSCs targeted therapy has been developed for treatment of breast cancer. BCSCs biomarkers play vital roles in early diagnosis, treatment and prognosis. The markers can be utilized for identification and isolation of BCSCs. They can also be used as a target to develop anticancer drugs. We have analyzed mechanisms of BCSCs resistance to conventional therapies. It was attributed to the ABC transporters, ALDH activity, inhibition of apoptosis and DNA repair, which could enhance anticancer drug extracellular transportation, maintain stemness, cellular detoxification, increase of survival rate in hostile environment, leading to chemoresistance and radioresistance of BCSCs. As we know, there is a close relationship between signaling pathway in BCSCs and its surrounding microenvironment. The cytokines in microenvironment are key mediators either to active or to inhibit the signaling transduction pathways in BCSCs. In turn, as a component of microenvironment, BCSCs variation could also induce alteration of its surrounding microenvironment. Understanding the regulation mechanisms between microenvironment and signaling pathways in BCSCs would be useful for us to add our knowledge about the tumor initiation and growth, which could be new therapeutic targets for the treatment of breast cancer. In addition, the development of miRNA transcriptome could be utilized for the regulation of BCSCs, which would also provide new targets for BCSC target therapy. Despite of some agents, such as salinomycin, lapatinib, curcumin, aromatase inhibitor and oncolytic virus have been developed and applied in clinic, more potential therapeutic strategies based on BCSCs needed to be further developed.

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