Advances in understanding the role and mechanisms of tumor stem cells in HER2-positive breast cancer treatment resistance (Review)

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Abstract. Approximately 15-20% of breast carcinomas exhibit human epidermal growth factor receptor (HER2) protein overexpression. HER2-positive breast cancer (BC) is a heterogeneous and aggressive subtype with poor prognosis and high relapse risk. Although several anti-HER2 drugs have achieved substantial efficacy, certain patients with HER2-positive BC relapse due to drug resistance after a treatment period. There is increasing evidence that BC stem cells (BCSCs) drive therapeutic resistance and a high rate of BC recurrence. BCSCs may regulate cellular self-renewal and differentiation, as well as invasive metastasis and treatment resistance. Efforts to target BCSCs may yield new methods to improve patient outcomes. In the present review, the roles of BCSCs in the occurrence, development and management of BC treatment resistance were summarized; BCSC-targeted strategies for the treatment of HER2-positive BC were also discussed.

Contents

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1. Introduction

Breast cancer (BC) has gradually replaced lung cancer as the most prevalent cancer type (1). BC is divided into four subtypes based on the expression status of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2) and antigen Ki-67 detected by immunohistochemistry: Luminal A, Luminal B, HER2-positive and triple-negative subtypes (2,3). Luminal A BC (ER+ and/or PR+, and HER2-, Ki-67 <14%) is characterized by high differentiation, slow growth and the best prognosis (4). Luminal B BC (ER+ and/or PR+, and HER2+ or HER2-, Ki-67 >14%) is sensitive to endocrine therapy and has a good prognosis (5). Triple-negative BC (ER- and PR-, and HER2-) is associated with short overall survival (OS) and unfavorable prognosis (6). HER2-positive BC (ER- and PR- and HER2+) is characterized by high aggressiveness, poor prognosis and chemotherapeutic resistance (7,8). HER/erythroblastic leukemia viral oncogene homolog (ERBB) is a member of the receptor tyrosine kinase signaling family, which includes HER1/ERBB1, HER2/ERBB2, HER3/ERBB3 and HER4/ERBB4 (9). In numerous types of malignant tumor, HER/ERBB family members exhibit overexpression, amplification or mutation, with effects on cell proliferation, migration, differentiation and apoptosis (10-13). HER2-positive BC is attributed to ERBB2/neu amplification or HER2 transmembrane receptor protein overexpression, which affects 15-20% of patients with BC (14). Currently, trastuzumab is the primary treatment; other targeted drugs [e.g., pertuzumab, neratinib, trastuzumab emtansine (T-DM1) and trastuzumab deruxtecan (T-DXd)] are also used in clinical treatment (15-18). However, drug resistance occurs in numerous patients after treatment (19). Cancer cell escape from drug treatment is related to the activities of BC stem cells (BCSCs), which exhibit

properties of self-renewal, infinite proliferation and multidirectional differentiation capacities necessary for the metastasis and recurrence of BC (20). In the present review, therapies for HER2-positive BC and the roles of BCSCs in HER2-positive BC treatment resistance were discussed. Recent research concerning BCSCs and related signaling pathways that may serve as therapeutic targets were also summarized, with the intention of providing a basis for inhibiting tumorigenesis and the development of HER2-positive BC.

2. Current status of HER2-positive BC treatment

In the clinical treatment of HER2-positive BC, three main types of drugs are used: Monoclonal antibodies (e.g., trastuzumab and pertuzumab), small-molecule tyrosine kinase inhibitors (TKIs; e.g., lapatinib, neratinib and tucatinib), and antibody-drug conjugates (T-DM1 and T-DXd) (Table I) (21).

Trastuzumab, a humanized antibody that acts on the extracellular domain IV region of the HER2 receptor (22), has demonstrated robust efficacy in the targeted treatment of HER2-positive BC over the past 20 years (23). Trastuzumab promotes cell apoptosis by inhibiting HER2 exocytosis, blocking the PI3K/AKT pathway and activating antibody-dependent cytotoxicity (24). Pertuzumab acts on region II of the HER2 receptor to block ligand-dependent HER2 heterodimer formation, thereby reducing HER2 intracellular signaling and inhibiting the proliferation and invasion of tumor cells (25).

Lapatinib is a dual-target TKI that acts on HER1/2 to inhibit the activation of downstream effectors (MAPK and AKT), leading to cell growth arrest and the acceleration of tumor cell regression (26,27). In addition, neratinib is an oral irreversible inhibitor that acts on HER1/2/4 to suppress the phosphorylation of MAPK and AKT, thereby attenuating cancer cell proliferation (28). Neratinib has been proven to inhibit the trastuzumab-induced upregulation of HER4 and enhance sensitivity to trastuzumab by limiting the activity of HER4 tyrosine kinase (29). Furthermore, neratinib may improve the 2-year disease-free survival in patients with early-stage HER2-positive BC (30). Another reversible, highly selective TKI is tucatinib, which acts on the intracellular tyrosine kinase region of the HER2 receptor (31) to inhibit signal transduction downstream of HER2/3 via the MAPK and PI3K/AKT pathways (32). A phase III trial indicated that tucatinib plus capecitabine and trastuzumab significantly prolonged progression-free survival and overall survival in patients with HER2-positive BC (33).

T-DM1 is an antibody-drug conjugate formed by conjugating trastuzumab to the cytotoxic drug emtansine (i.e., DM1) using a linker (34). T-DM1 retains trastuzumab activity and simultaneously induces apoptosis by delivering the microtubule inhibitor DM1 to HER2-overexpressing tumor cells (35). T-DXd is a novel antibody-drug conjugate composed of trastuzumab and the topoisomerase type I inhibitor DXd using a linker (36). T-DXd has a high drug-to-antibody ratio and favorable membrane permeability. In addition, DXd may induce DNA fragmentation. Thus, T-DXd exhibits a robust killing effect on HER2-overexpressing tumor cells (37).

Although various targeted drugs are effective, numerous patients subsequently exhibit primary or acquired drug resistance, leading to accelerated disease progression (38). Thus, there is considerable interest in identifying effective therapies for the management of drug resistance.

3. BCSCs and related signaling pathways

BCSCs. CSCs were first confirmed in early studies of leukemia models (39). CSCs have the capacity to self-renew, differentiate and promote tumorigenic development (40). BCSCs were first identified in a xenograft solid tumor in 2003, which caused malignant proliferation, invasion, metastasis and recurrence of BC (41). Accumulating evidence has indicated the association between trastuzumab resistance and BCSCs in HER2-positive BC (42,43). BCSC-targeted therapy may be a promising way to counteract trastuzumab resistance.

BCSC phenotypes. BCSCs may be characterized by the distribution of biomarkers on the cell membrane, such as CD44, CD24, acetaldehyde dehydrogenase (ALDH)1 and CD133 (44). The membrane glycoproteins CD44 and CD24 are promising BCSC biomarkers. CD44 interacts with its primary ligand hyaluronic acid to activate various signaling pathways, which participate in cell proliferation and invasion (45,46). Due to its rarity, CD24 expression in BCSCs is usually assessed in combination with CD44 expression. The $CD44^+/CD24^{-/low}$ phenotype is a classical BCSC biomarker that may be used to assess distant metastasis, recurrence and prognosis (47). Furthermore, the plasticity of BCSCs enables them to switch between epithelial-mesenchymal transition (EMT, mesenchymal-like state) and mesenchymal-epithelial transition (epithelial-like state), leading to tumor invasion and metastasis (48). It has been reported that mesenchymal-like $CD44^+/CD24^{-/low}$ cells may be responsible for the resistance of HER2-positive BC to trastuzumab (49). ALDH1, a cellular lipase present in cells capable of self-renewal and multilineage differentiation, is an important BCSC biomarker (50). Liu *et al* (51) demonstrated that ALDH1 expression was positively correlated with breast tumor growth. BCSCs exhibit dormant and proliferative states; dormant BCSCs are more resistant to antimitotic drugs (52). Another study indicated that mesenchymal BCSCs with high $CD44^+/CD24^-$ expression were in the dormant state, whereas epithelioid BCSCs with high $ALDH^+$ expression were in the proliferative state (48). In the past 10 years, $CD44^+CD24^{-/low}$ $ALDH^+$ expression has been used as a specific BCSC biomarker, particularly for HER2-positive BC (53). The population of $CD44^+/CD24^{-/low}$ phenotype BCSCs significantly increases in HER2-positive MDA-MB-435 cells than other cell lines (54). $CD133^+$, also known as prominin-1, is associated with poor prognosis, angiogenesis, lymph node metastasis and HER2 positivity in BC (55,56). $EPHA5^-$, a receptor tyrosine kinase, is able to increase BCSC properties and increase the resistance of HER2-positive BC to trastuzumab (56). Collectively, BCSC phenotypes are closely connected to the development of HER2-positive BC. Thus, specific phenotypic BCSC-targeted therapies may be a promising approach to overcome BC and treatment resistance.

BCSC-related signaling pathways. Several signaling pathways are involved in shaping the properties of BCSCs, including the Wnt/β-catenin, Notch and Hedgehog pathways. Pathway dysregulation or aberrant activation induces abnormal BCSC proliferation, leading to reduced sensitivity to drug therapy

Table I. Current targeted drugs and mechanisms of action for HER2-positive breast cancer.

Type/drug	Target	Mechanism of action	(Refs.)
Monoclonal antibodies			(22,24)
Trastuzumab	HER2	Inhibition of exocytosis of HER2 Blocking of the PI3K/AKT signaling pathway ADCC	
Pertuzumab	HER2	Blocking of ligand-dependent HER2 heterodimer formation Reduction of HER2 intracellular signaling	(25)
TKIs			
Lapatinib	HER1 HER2	Inhibition of MAPK and AKT activation	(27)
Neratinib	HER1 HER2 HER4	Inhibition of MAPK and AKT phosphorylation	(28,29)
Tucatinib	HER2 HER3	Inhibition of MAPK and PI3K/AKT pathways	(31,32)
ADC			
T-DM1	HER2	Inhibition of tubulin polymerization Retainment of trastuzumab activity Delivery of DM1 to tumor cells	(34,35)
T-Dxd	HER2	Retainment of trastuzumab activity Induction of DNA fragmentation	(36,37)

ADCC, antibody-dependent cytotoxicity; TKIs, tyrosine kinase inhibitors; MAPK, mitogen-activated protein kinases; ADC, antibody-drug conjugate; T-DM1, trastuzumab emtansine; T-DXd, trastuzumab deruxtecan; HER2, human epidermal growth factor receptor.

and enhancement of BC development (Fig. 1). Thus, a deep understanding of these pathways may lead to the discovery of novel targeted therapies.

The Wnt/β-catenin signaling pathway is associated with the proliferation, migration and chemotherapeutic resistance of BCSCs. Wnt proteins usually bind to Frizzled receptors (i.e., G-protein-coupled receptors) and low-density-lipoprotein receptor-related protein 5 or 6 (LRP5/6) to form Wnt-FZD-LRP5/6 trimeric complexes in an autocrine or paracrine manner, leading to catenin stabilization (Fig. 1A) (57). Activation of the Wnt/β-catenin pathway promotes EMT, treatment resistance and self-renewal in BCSCs (58). Wu *et al* (59) suggested that upregulated Wnt3 activated the Wnt/β-catenin signaling pathway that may lead to trastuzumab resistance in HER2-positive BC cells. Cyclin-dependent kinase 12 may induce proliferation and tumor recurrence in BCSCs through effects on the Wnt/β-catenin pathway, leading to low trastuzumab efficacy in the treatment of HER2-positive BC (60). Furthermore, high RNA expression levels of Wnt in BCSCs led to an increased metastatic rate and shortened the overall survival of patients (61).

The Notch signaling pathway has four receptors (Notch1-4) and five associated ligands [Jagged-1-2 and Delta-like ligand (DLL)-1-4] (Fig. 1B) (62). The Notch pathway is closely associated with BC occurrence and progression. Through ligand-receptor binding interactions, aberrant Notch activation promotes aggressiveness and drug resistance in BCSCs. Baker *et al* (63) found that Notch-1 maintained BCSC survival by inhibiting phosphatase and tensin homolog, which led to drug resistance in HER2-positive BC cells. Pandya *et al* (64) reported that protein kinase C-α reversed trastuzumab resistance in HER2-positive BC by inhibiting Jagged-1-mediated notch signaling.

The Hedgehog signaling pathway consists of three ligands (Sonic, desert and Indian hedgehog), two receptors [Patched (PTCH) and smoothened (SMO)], and the glioma-associated oncogene transcription factors (GLI)1-3 (Fig. 1C). He *et al* (65) found that PTCH, SMO, GLI1 and GLI2 were significantly upregulated in BCSC-enriched MCF-7 mammosphere cells. High GLI1 expression is associated with trastuzumab resistance and poor prognosis in HER2-positive BC (66). Gupta *et al* (67) demonstrated that silencing of the GLI2 gene inhibited HER2-positive BC invasion and metastasis. Doheny *et al* (68) reported that knockdown of SMO inhibited BCSC growth, suggesting that Hedgehog pathway inhibitors may be useful in BCSC-targeted therapy. In addition, further signal transduction pathways, including the Hippo (69), TGF-β (70), JAK2/STAT3 (71) and PI3K/AKT/mTOR (72) pathways, are closely associated with BCSCs through their effects on BC occurrence and progression.

4. Mechanism of BCSCs involvement in HER2-positive BC resistance

Increasing evidence has indicated that BCSCs accelerate BC progression due to their stem cell properties, drug resistance

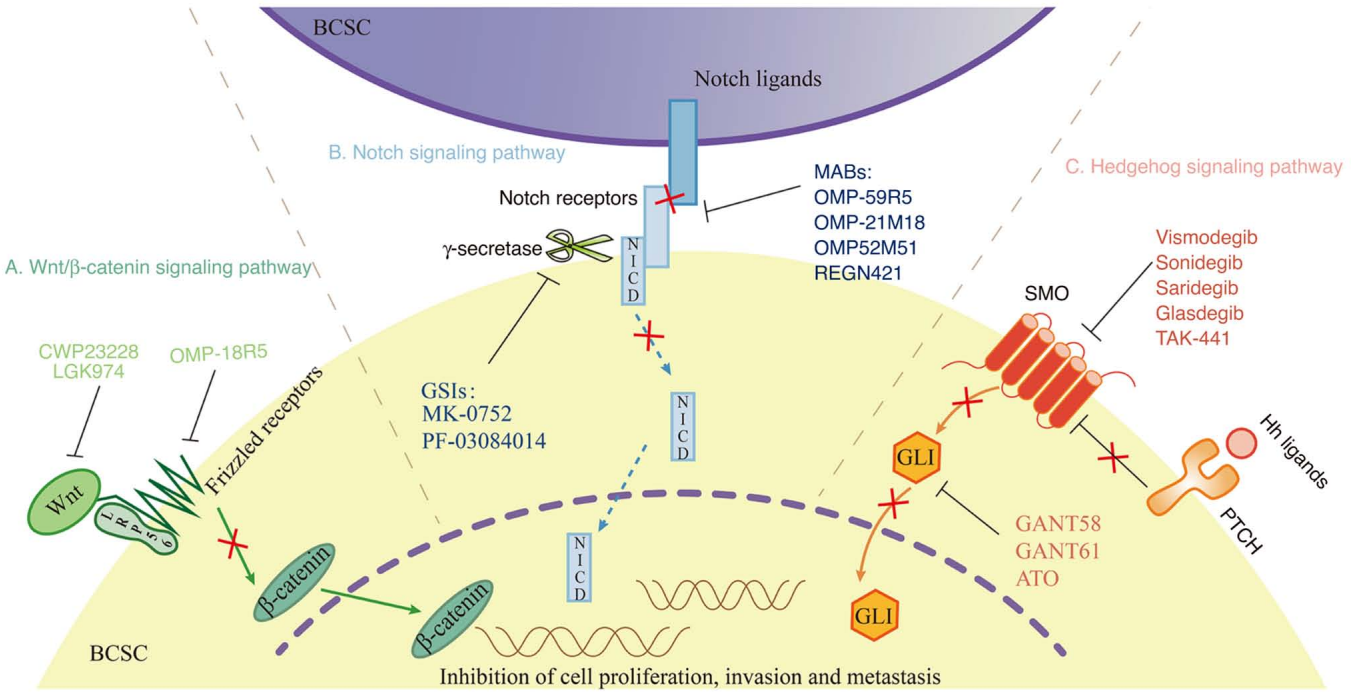

Figure 1. BCSC-related signaling pathways and their inhibitors. (A) Wnt signaling pathway and its inhibitors. The trimer composed of Wnt protein, frizzled receptors and LRP5/6 receptors mediates the stable expression of β-catenin, forming the classic Wnt/β-catenin signaling pathway. The inhibitors of this pathway include Wnt protein inhibitors and Frizzled receptors inhibitor. (B) Notch signaling pathway and its inhibitors. The Notch signaling pathway is activated when the Notch receptor binds to ligands on adjacent cell membranes. Subsequently, γ-secretase is responsible for cutting the proteins in the transmembrane domain and releasing the NICD into the cytoplasm. NICD eventually enters the nucleus and regulates the transcriptional activity of target genes. Inhibitors of this pathway include GSIs and MABs. (C) Hh signaling pathway and its inhibitors. Binding of Hh ligands to PTCH results in SMO disinhibition, which leads to activation of GLIs. Activated GLIs enter the nucleus and promote target gene transcription. The main inhibitors of this pathway are SMO inhibitors and GLI inhibitors. The above signaling pathway inhibitors are effective therapies targeting BCSCs, which may inhibit proliferation, invasion and metastasis of BCSCs. BCSCs, breast cancer stem cells; Hh, hedgehog; GSIs, γ-secretase inhibitors; MABs, monoclonal antibodies; NICD, Notch intracellular domain; LRP5/6, LDL receptor-related protein 5 or 6; PTCH, Patched receptor; SMO, smoothened receptor; GLIs, glioma-associated oncogenes.

and immune evasion (73). In the following chapter, the mechanisms of the involvement of BCSCs in the treatment resistance of HER2-positive BC is discussed. There are several possible mechanisms BCSCs participate in to induce HER2-positive BC resistance, including the tumor microenvironment, ABC transporters and non-coding RNAs.

BCSC microenvironment affects BCSC drug resistance. The BCSC microenvironment mainly consists of cytokines, the extracellular matrix (ECM), vascular microenvironment and bone marrow microenvironment. Cytokines (e.g., IL-6, IL-8 and TGF-β), are secreted by cancer-associated fibroblasts (CAFs), endothelial cells (ECs), mesenchymal stem cells (MSCs) and tumor-associated macrophages, regulating drug resistance by activating BCSC-related signaling pathways (74-76). Mao *et al* (77) demonstrated that CAFs induce trastuzumab resistance by secreting IL-6 to expand BCSCs and activate multiple pathways in HER2-positive BC (Fig. 2A). The ECM forms a protective membrane at the periphery of a cluster of cancer cells; this physical barrier weakens drug penetration and protects BCSCs from drug elimination (78). Collagen is the main structural protein in the ECM, and collagen type I α1 (COL1A1) promotes cell proliferation and drug resistance in BC (Fig. 2B) (79). Hanker *et al* (80) indicated high COL1A2 expression was related to lower clinical response to trastuzumab by regulating PI3K/AKT signaling in patients with HER2-positive BC. The ECM also regulates the ability of BCSCs to boost growth and survival, thereby contributing to therapeutic resistance (81). Through their multidirectional differentiation potential, BCSCs may differentiate into ECs, which allows participation in angiogenesis and alteration of the vascular microenvironment (Fig. 2C) (82). Hori *et al* (83) found that HER2-positive BC cells exhibit vasculogenic mimicry in the angiogenic microenvironment after complete trastuzumab resistance. Additional studies have demonstrated that increased expression of stemness markers, such as octamer-binding transcription factor 4 (Oct4), aldehyde dehydrogenase 1 (ALDH1) and CD44 in BCSCs promote BC cell growth and treatment resistance (84-86). In the bone marrow microenvironment, extracellular vesicles released from MSCs may be internalized by BCSCs, promoting drug resistance in BC cells (87). Kim *et al* (88) reported that the IL-6-JAK1-STAT3-Oct-4 signaling pathway in the bone marrow microenvironment was able to convert non-BCSCs into BCSCs by regulating BCSC-associated Oct-4 gene expression. In addition, the hypoxia environment increased the population of BCSCs and induced trastuzumab resistance in HER2-positive BC cells (89,90). Lee *et al* (91) found that hypoxia-inducible factor-1α promoted BCSC aggregation and tumor recurrence. Furthermore, the expression levels of multiple BCSC biomarkers [e.g., ATP-binding cassette G member 2 (ABCG2), sex-determining region Y-box 2, Krüppel-like factor 4 and $CD44^{+}/CD24^{-/low}$] are upregulated under hypoxic conditions, contributing to increased drug resistance in BC cells (Fig. 2D) (92).

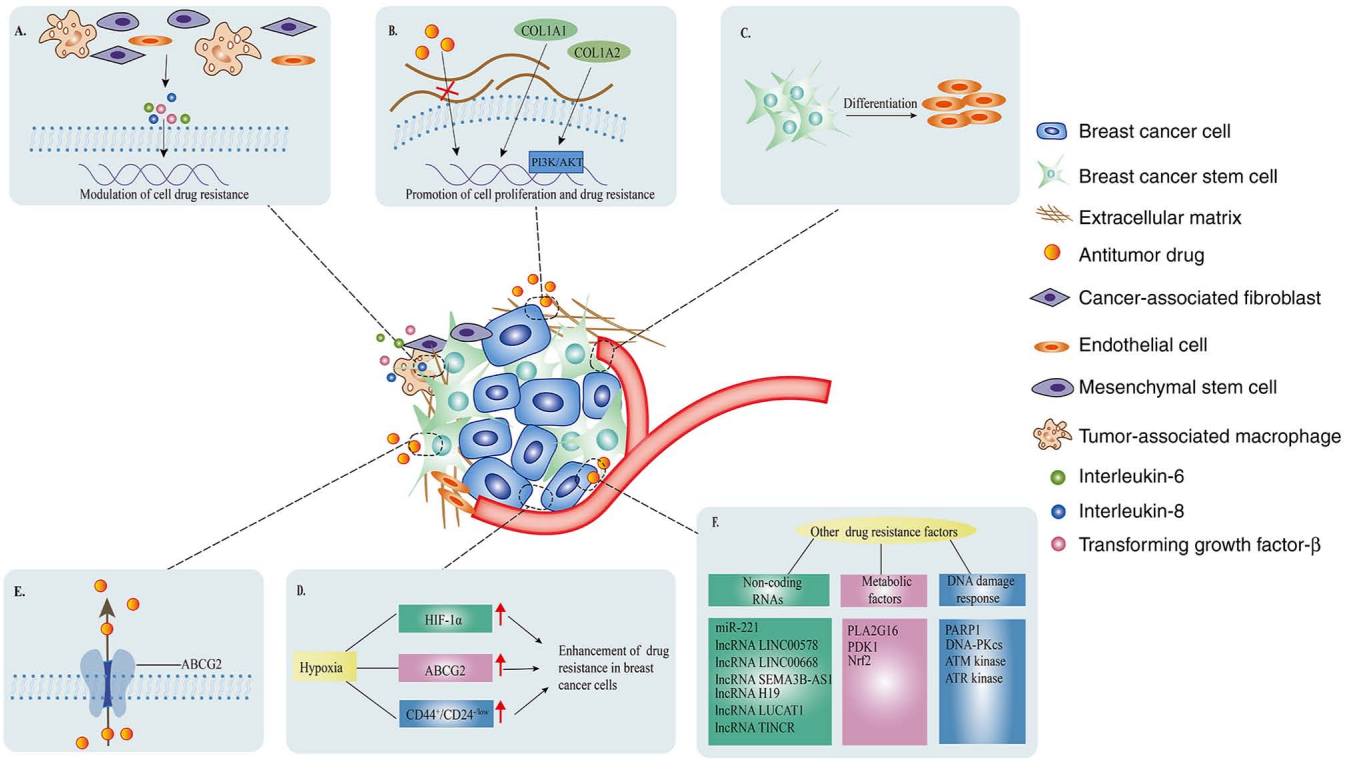

Figure 2. Mechanism of BCSCs in human epidermal growth factor receptor-positive BC resistance. (A) Cancer-associated fibroblasts, endothelial cells, mesenchymal stem cells and tumor-associated macrophages secrete a variety of cytokines to regulate cellular drug resistance. (B) The presence of extracellular matrix may weaken the penetration of anticancer drugs into cells and its main structural protein COL1A1/2 may promote cell proliferation and drug resistance. (C) BCSCs differentiate into vascular endothelial cells. (D) In the hypoxic environment, increased expression of HIF-1α, ABCG2, $CD44^{+}/CD24^{-/low}$ led to drug resistance of BCSCs. (E) ABC transporter mechanism. ABCG2 is one of the ABC transporters, which may excrete anticancer drugs and lead to drug resistance in cells. (F) Other drug resistance factors such as ncRNAs, metabolic factors and DNA damage response. BCSCs, breast cancer stem cells; CAFs, cancer-associated fibroblasts; COL1A1/2, collagen type I α1/2; ABC, ATP-binding cassette; ABCG2, ABC cassette G member 2; HIF, hypoxia-inducible factor; lncRNA, long noncoding RNA; miR, microRNA.

Drug pump effect of ABC transporter facilitates drug resistance among BCSCs in HER2-positive BC. ABC transporter overexpression is an important factor that contributes to multidrug resistance in HER2-positive BC (93). Through the drug discharge pump mechanism, ABC transporters mediate intracellular drug outflow and help to decrease intracellular drug concentrations, thereby enhancing drug resistance in BCSCs (94). ABCG2, a representative member of the ABC transporter family, has a vital role in the development of multidrug resistance in HER2-positive BC (Fig. 2E) (95). Němcová-Fürstová *et al* (96) indicated higher expression of ABCG2 protein in paclitaxel-resistant SK-BR-3 cells. Furthermore, inhibition of the Wnt pathway may attenuate ABCG2 expression (97). Overall, the drug pump effects of ABC transporters facilitate drug resistance among BCSCs in HER2-positive BC.

Other important drug resistance factors. In the past 10 years, the involvement of non-coding RNAs in HER2-positive BC resistance via regulation of BCSCs (Fig. 2F) has attracted considerable attention. Ye *et al* (98) found that microRNA (miR)-221 was able to induce BCSC proliferation, thereby reducing the sensitivity of HER2-positive BC to drug therapy. Elevated expression of long non-coding RNAs [lncRNAs; e.g., LINC00578, LINC00668 and SEMA3B antisense RNA 1 (SEMA3B-AS1)] in HER2-positive BC enhanced BCSC stemness (99). In addition, the expression levels of lncRNA H19 (100), lung cancer-associated transcript 1 (101) and terminal differentiation-induced non-coding RNA (102) were observed to be higher in HER2-positive BC tissues than in normal breast tissues. Conversely, miR-375 and lncRNA growth-arrest-specific 5 attenuated the proliferation and drug resistance capacities of tumor cells (103,104). Numerous metabolic factors are associated with BCSC involvement in HER2-positive BC resistance. For instance, group XVI phospholipase A2, a promoter associated with phospholipid metabolism, contributes to the maintenance of BCSC characteristics and may serve as a BCSC biomarker (105). Pyruvate dehydrogenase kinase 1, produced during glycolysis, significantly increases the numbers of $ALDH^{+}$ BCSCs and promotes BC progression (106). Fox *et al* (107) found that targeted HER2 therapy led to the activation of nuclear factor erythroid 2-related factor 2 in dormant tumor cells by modulating redox potential and nucleotide metabolism. DNA damage repair (DDR) is a prevalent phenomenon in BCSCs, where it facilitates repair after reactive oxygen species-mediated damage to DNA (108,109). Overexpression of poly[ADP-ribose] polymerase 1 was reported to enhance tolerability to DNA damage in trastuzumab-resistant HER2-positive BC (110). In addition, certain DNA damage sensor proteins, such as the DNA-dependent protein kinase catalytic subunit, the ataxia-telangiectasia-mutated kinase and the ataxia-telangiectasia

and Rad3-related kinase, are also involved in the DDR (111). Therefore, the inhibition of DDR signaling may enhance BCSC sensitivity to chemotherapy and substantially improve the prognosis of patients.

5. BCSC-targeted therapeutic strategies

Wnt/β-catenin signaling pathway inhibitors. Porcupine is a critical enzyme involved in Wnt ligand secretion and acylation (112). LGK974 (Wnt974) is a specific membrane-bound porcupine inhibitor that suppresses the Wnt/β-catenin signaling pathway, thereby inhibiting BCSC self-renewal and migration (113). Jang *et al* (114) reported that cwp232228, a small-molecule inhibitor, impaired the growth of BCSCs and BC cells by blocking the Wnt/β-catenin pathway; the inhibitory effect was more noticeable in BCSCs than in BC cells. In addition, OMP-18R5, a monoclonal antibody targeting the Wnt pathway by blocking Frizzled receptors, provides an efficacious approach for BC treatment (115). Mu *et al* (116) reported that dickkopf-associated protein 2 induced apoptosis in BCSCs by regulating the Wnt signaling pathway. Furthermore, numerous Wnt inhibitors have been used in preclinical studies. For instance, salinomycin limits BC invasiveness and reduces BCSC resistance to drug treatment (117).

Notch signaling pathway inhibitors. Mutations in the Notch signaling pathway regulate the development of drug resistance among BCSCs. There are two main types of Notch inhibitor: Notch receptor cleavage inhibitors [e.g., γ-secretase inhibitors (GSIs)] and monoclonal antibodies that interfere with receptor-ligand binding. GSIs mainly include MK-0752 and PF-03084014. MK-0752 and PF-03084014 have demonstrated good efficacy in clinical trials on the treatment of advanced BC (118,119). Treatment with GSIs plus docetaxel led to a reduction in the number of BCSCs, downregulation of $CD44^+/CD24^-$ and $ALDH^+$ biomarkers and a decrease in BC volume (118). Monoclonal antibodies against Notch receptors or ligands include OMP-59R5, OMP-21M18, OMP-52M51 and REGN421, and their targets are Notch2/3, DLL-4, Notch1 and DLL-4, respectively (119). These drugs enhance antitumor activity when combined with typical targeted agents (120-123). Li *et al* (56) demonstrated that erythropoietin-producing hepatocellular receptor A5 inhibited BCSC self-renewal via the Notch1 signaling pathway, thereby reducing the risk of trastuzumab resistance in HER2-positive BC.

Hedgehog signaling pathway inhibitors. Hedgehog signaling pathway inhibitors may be categorized as SMO inhibitors (vismodegib, sonidegib, saridegib, glasdegib and TAK-441) and GLI inhibitors (GANT58, GANT61 and arsenic trioxide) (124). Vismodegib and sonidegib have been approved by the Food and Drug Administration for the therapy of metastatic or recurrent basal cell carcinoma; they significantly inhibit the spread of metastatic cells and improve median patient survival (125). GANT58 and GANT61 are also in preclinical studies (126). Liu *et al* (127) reported that cordycepin inhibited SMO receptors and GLI transcription factors, thereby limiting BC cell growth and metastasis. Although several inhibitors remain in the preclinical stage of investigation, these new approaches may enhance the effectiveness of BCSC-targeted resistance (128).

Other pathway inhibitors. TAZ and YAP, two core transcription factors in the Hippo signaling pathway, have essential roles in BC occurrence and development. Inhibitors targeting TAZ/YAP may restrict BCSC proliferation and tumorigenesis. Statins may inhibit TAZ/YAP activity and block signaling transduction in the Hippo pathway (129). Furthermore, numerous preclinical studies on TGF-β inhibitors are underway, including the investigation of recombinant RNA technology that may interfere with TGF-β signaling to inhibit the proliferation and invasion of BC cells (130). In addition, Wang *et al* (131) demonstrated that inhibition of the JAK2/STAT3 pathway led to downregulation of the expression of key fatty acid β-oxidation enzymes in BCSCs, restoring their sensitivity to chemotherapy. As drugs that target a single signaling pathway may be insufficient for clinical needs, diverse multitarget strategies are required for future treatment of HER2-positive BC.

Therapies targeting BCSC status. Dormant BCSCs may evade drug treatment and undergo plastic transformation with proliferating cells, leading to recurrence and metastasis. Non-coding RNAs may be involved in converting dormant BCSCs into proliferative BCSCs (132). LncRNA-Na^+-sulfate cotransporter 1 is upregulated in dormant mesenchymal-like BCSCs, where it contributes to a prolonged dormancy period and reduces tumorigenicity (133). Similarly, the combined effects of Src family kinase inhibitors and MEK1/2 inhibitors may extend dormancy in BCSCs and induce apoptosis to prevent BC recurrence (134). In addition, a Tet methylcytosine dioxygenase 2-targeted strategy was observed to be able to transform dormant cells into active proliferating cells, thus restoring chemotherapeutic sensitivity (135). Therefore, therapeutic exploitation of BCSC status involves directly eliminating dormant cells or suppressing cell transition from dormancy to proliferation.

Therapies targeting BCSC microenvironment. As mentioned above, the BCSC microenvironment participates in the onset of treatment resistance; therefore, strategies targeting the BCSC microenvironment may be useful. COL1A1 knockdown reduces cell proliferation and invasion, leading to decreased expression of stemness markers (e.g., sex-determining region Y-box2, octamer-binding transcription factor 4 and CD133) that inhibit EMT and stem cell activity (136). Furthermore, abnormalities in the vascular microenvironment may hinder therapeutic effects. Chen *et al* (137) indicated that erlotinib was able to normalize the tumor vascular system, improve perfusion and oxygenation, and enhance the chemotherapeutic effects of nanodrugs in a mouse model of BC. In addition, Kim *et al* (138) reported that AzCDF, a small molecule drug, was able to target BCSCs in a hypoxic environment, blocking tumor growth and lowering tumorigenesis rates. The inhibition of TGFβ-inducible protein expression improved hypoxia and tumor angiogenesis, thereby reducing the number of BCSCs and inhibiting cancer cell metastasis (139).

ABC transporter inhibitors. Apatinib significantly downregulates the expression of ABCG2 to inhibit BCSC proliferation (140). Wu *et al* (141) demonstrated that progesterone increased BCSC sensitivity to drug treatment by

modulating ABCG2 transcriptional activity, which led to decreased drug efflux. Lapatinib was found to block ABCG2-mediated efflux in HER2-positive BC cells (142). Elacridar, the ABCG2-transporter inhibitor, enhances the therapeutic effect of lapatinib on HER2-positive advanced and metastatic BC (143). Yi *et al* (144) indicated that pyrotinib was able to inhibit the expression of ABCG2 to restore the sensitivity of drug-resistant HER2-positive BC cells.

Other special drugs. Metformin, an anti-diabetes drug, is able to selectively kill BCSCs by inhibiting the PI3K/AKT/mTOR pathway and improving BC sensitivity to drug therapy (105,145,146). The expression levels of IL-8 were positively associated with BCSC activity; inhibition of the chemokine receptors C-X-C motif chemokine receptor (CXCR)1/2 was able to reduce the level of IL-8 (147). Therefore, small molecule antagonists of CXCR1/2, in combination with HER2-targeted therapy, have the potential to inhibit BCSC activity and prolong the survival of patients with HER2-positive BC (148). The DDR is activated to repair DNA damage in BCSCs and ATR is a major regulator of the DDR. Kim *et al* (149) demonstrated that AZD6738, an ATR inhibitor, considerably reduced DDR efficiency and weakened BCSC formation in HER2-positive BC. Several types of DDR inhibitors are currently in development.

6. Conclusions and perspectives

Increasing evidence indicates that BCSCs have critical roles in the treatment resistance and recurrence of HER2-targeted therapy. An improved understanding of the mechanism by which BCSCs contribute to drug resistance will help to prevent breast tumor recurrence and drug resistance. The mechanism of drug resistance of BCSCs is complex. Factors such as abnormal signaling pathway activation, BCSC microenvironment, ABC transporters and BCSC repair capacity may lead to BCSC proliferation and the onset of drug resistance in HER2-positive BC. The development of BCSCs-targeted treatment approaches is expected to improve the effectiveness of HER2-positive BC. A variety of therapeutic strategies have been implemented to eliminate or reduce BCSCs, which may restore trastuzumab sensitivity *in vitro* and *in vivo*. However, most of the therapies are still restricted to laboratory investigation. Therefore, in future studies, it is necessary to clarify new biological characteristics and molecular mechanisms of BCSCs and develop combination therapy or multi-target therapy to overcome and reverse the drug resistance of BCSCs, ultimately improving the cure rate and reducing the recurrence rate of HER2-positive BC.

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Authors' contributors

LX and QL were responsible for the conceptualization, methodology and writing-original draft preparation. FH and XS were responsible for supervision, writing-reviewing and editing and funding acquisition. LZ, WD, KZ, CK and NH were responsible for data curation and investigation. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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