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REVIEW

Nanosized Drug Delivery Systems for Breast Cancer Stem Cell Targeting

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Abstract: Breast cancer stem cells (BCSCs), also known as breast cancer initiating cells, are reported to be responsible for the initiation, progression, therapeutic resistance, and relapse of breast cancer. Conventional therapeutic agents mainly kill the bulk of breast tumor cells and fail to eliminate BCSCs, even enhancing the fraction of BCSCs in breast tumors sometimes. Therefore, it is essential to develop specific and effective methods of eliminating BCSCs that will enhance the efficacy of killing breast tumor cells and thereby, increase the survival rates and quality of life of breast cancer patients. Despite the availability of an increasing number of anti-BCSC agents, their clinical translations are hindered by many issues, such as instability, low bioavailability, and off-target effects. Nanosized drug delivery systems (NDDSs) have the potential to overcome the drawbacks of anti-BCSC agents by providing site-specific delivery and enhancing of the stability and bioavailability of the delivered agents. In this review, we first briefly introduce the strategies and agents used against BCSCs and then highlight the mechanism of action and therapeutic efficacy of several state-of-the-art NDDSs that can be used to treat breast cancer by eliminating BCSCs.

Keywords: breast cancer stem cells, nanosized drug delivery systems, BCSCs, NDDSs, surface markers, signaling pathway, target

Introduction

Despite many advances in breast cancer therapy with the development of drugs targeting breast cancer cells, breast cancer still remains one of the major causes of patient deaths worldwide.[1] Therapeutic resistance, recurrence, and metastasis are the leading challenges in breast cancer treatment.[2] Accumulating evidence has demonstrated that breast cancer stem cells (BCSCs), also known as breast cancer initiating cells, are responsible for the poor prognosis of breast cancer; as they play key roles in the initiation, progression, therapeutic resistance, and recurrence of breast cancer.[3–8] BCSCs possess specific markers that distinguish them from bulk tumor cells, such as the high expression of surface antigen cluster of differentiation 44/low or negative expression of cluster of differentiation 24 ($CD44^{+}/CD24^{low/-}$),[9] high expression of CD133 ($CD133^{+}$)[10] and positive expression of aldehyde dehydrogenase 1 ($ALDH1^{+}$).[11] Moreover, BCSCs are intrinsically drug resistant and often display high expression levels of drug efflux transporters and over-activation of anti-apoptotic signaling pathways.[12–14] Besides, breast cancer treatment with conventional chemotherapy or radiotherapy can kill only bulk tumor cells and fail to eliminate BCSCs, possibly even enhancing the fraction of BCSCs in breast tumors. These residual BCSCs will become cancer cells in the future, and

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consequently lead to tumor recurrence.[15–17] In addition, BCSCs facilitate the metastasis of breast cancer by inducing the transformation of epithelial cancer cells to mesenchymal cancer cells, allowing them to spread easily to distant lesions.[18] Therefore, it is crucial to eliminate BCSCs to enhance the efficacy of breast cancer treatment.

In recent years, many strategies have been proposed to eradicate BCSCs by blocking the signaling pathways related to BCSCs self-renewal such as Wnt/β-catenin,[19] Hedgehog (Hh),[20] Notch,[21] Hippo[22] and transforming growth factor-β (TGF-β).[23] Blockage of these pathways is designed to inhibit the proliferation and growth of breast tumors, target the breast tumor microenvironment to destroy the communication between BCSCs and cytokines,[24–26] target the BCSC surface markers to locate and destroy BCSCs,[27–30] and interfere with the differentiation[31] or metabolism[32,33] of BCSCs to render them more sensitive to conventional therapy. An increasing number of anti-BCSC agents have been proposed to treat breast cancer based on the above strategies, such as quercetin,[34] sulforaphane,[35] curcumin,[36] salinomycin (SAL),[37] nuclear factor-kappa B (NF-κB) short hairpin ribonucleic acid (shRNA),[38] octamer 4 (Oct-4) small interfering RNA (siRNA),[39] and microRNA-100 (miR-100).[6] However, similar to conventional chemotherapeutic drugs, most currently reported anti-BCSC agents have disadvantages such as poor solubility, low stability, high toxicity, unfavorable pharmacokinetics, and lack of tissue selective distribution,[3] that restrict their clinical applications. In addition, these agents are potentially toxic to normal stem cells as BCSCs share properties with normal stem cells and conventional anti-BCSC agents cannot distinguish them from normal stem cells.

Because of their site-specific delivery and enhanced drug stabilization, nanosized drug delivery systems (NDDSs) have shown significant promise in the delivery of anti-BCSC agents and have the potential to overcome the limitations of conventional anti-BCSC agents mentioned above. There are an increasing number of NDDSs used to deliver anti-BCSC agents, including polymeric nanoparticles,[40] micelles,[41] liposomes,[42] nanocomplexes,[38] nanoprodrugs,[43] aptamer–conjugated deoxyribonucleic acid (DNA) nanotrains,[16] single-walled carbon nanotube (SWCNT) nanocarriers,[44] nanoexosomes,[45] lipid–polymer hybrid nanoparticles,[46] and nanocages.[47] However, the clinical translation of these NDDSs is tough, as many challenges still remain unaddressed. In this review, we briefly introduce the strategies and agents used against BCSCs and then highlight the mechanism of action and therapeutic efficacy of several state-of-the-art NDDSs that can be used to treat breast cancer by eliminating BCSCs.

Current Treatment Strategies Against Breast Cancer by Inhibition of BCSCs

A growing body of research has shown that BCSCs account for breast cancer initiation, progression, recurrence, and therapeutic resistance. In addition, BCSCs can self-renew and give rise to non-tumorigenic cancer cells.[48,49] Therefore, it is necessary to completely eliminate BCSCs to successfully eradicate breast cancer. In recent years, many strategies to treat breast cancer by targeting BCSCs have been proposed, such as targeting BCSC surface markers,[27,28,50] inhibiting BCSC-dependent signaling pathways,[19–21,23] interfering with BCSC differentiation,[31,51] targeting metabolism in BCSCs,[52–54] and targeting the breast tumor microenvironment.[55,56] Therapeutic strategies against breast cancer according to the characteristics of BCSCs are shown in Figure 1.

Targeting BCSC Surface Markers

The surface markers on cancer stem cells play a crucial role in the isolation, identification, diagnosis, and targeted therapy of cancer stem cells. Commonly used surface markers of BCSCs include CD44, CD133, and epithelial cell adhesion molecule (EpCAM). CD44 is a transmembrane protein that has been identified in many cancer stem cells, including BCSCs, and it plays a very important role in regulating the properties of BCSCs that involve self-renewal, tumor initiation, therapeutic resistance, and metastasis.[27,50] The characteristic CD44 overexpression in BCSCs indicates that CD44 is a potential target in the treatment of BCSCs. Therefore, anti-CD44 with monoclonal antibodies or ligands may be promising strategies for eliminating BCSCs. P245, an anti-CD44 antibody, has been demonstrated to inhibit breast cancer growth and eliminate BCSCs in xenograft mouse models.[57] Treatment with P245 prevented tumor recurrence in human breast cancer xenografts after treatment with doxorubicin (DOX) and cyclophosphamide.[57] CD133, also known as prominin-1, is a five-transmembrane glycoprotein that is overexpressed in several types of cancers, such as breast cancer, ovarian cancer, and gastric carcinoma.[28,58] As one among several surface markers of BCSCs, CD133 is critical for the survival and growth of BCSCs, and antibodies against CD133

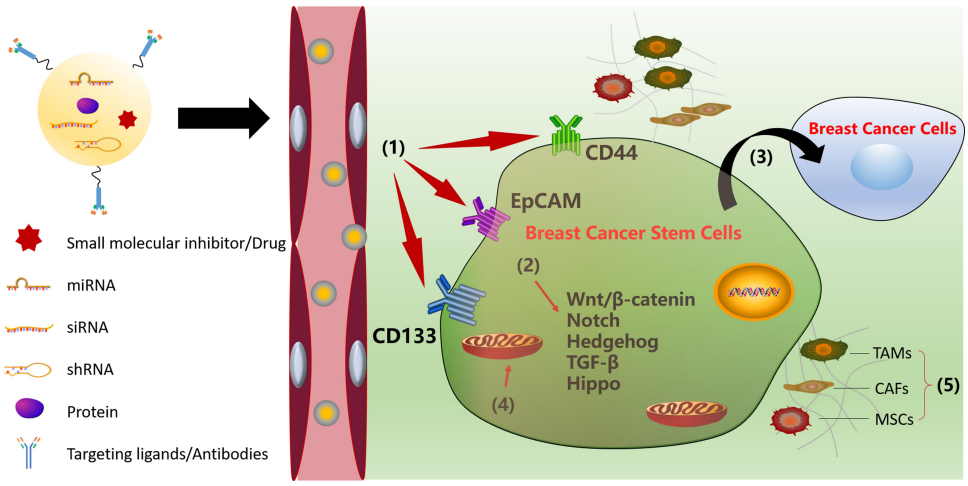

Figure 1 Strategies against breast cancer stem cells: (1) targeting BCSC surface markers; (2) inhibition of BCSC-dependent signaling pathways; (3) interfering the BCSC differentiation; (4) targeting metabolisms in BCSCs; (5) targeting the breast tumor microenvironments.
Abbreviations: TAMs, tumor-associated macrophages; CAFs, cancer-associated fibroblasts; MSCs, mesenchymal stem cells.

can reduce the growth of BCSCs. It was demonstrated that the fusion protein dCD133KDEL represents a new biological assessment tool that can be used to determine the clinical significance of eradicating $CD133^+$ cells.[59] EpCAM (also known as CD326/ESA), a glycoprotein, is another molecular target of BCSCs, and its overexpression may facilitate the proliferation, metastasis, and drug resistance of breast cancer cells.[29,30] Kubo et al[30] found that catumaxomab, an EpCAM antibody, combined with activated T-cells could eliminate EpCAM-positive triple-negative breast cancer cells and overcome the chemoresistance induced by these cells in vitro.

Inhibition of BCSC-Dependent Signaling Pathways

Dysregulation of signaling pathways plays an important role in rendering BCSCs capable of maintaining stem cell characteristics and facilitates the role of BCSCs in the development and progression of breast tumors.[60,61] The major signaling pathways related to the maintenance, self-renewal, survival, and differentiation of BCSCs are Wnt/β-catenin,[19] Hh,[20] Notch,[21] Hippo[22] and TGF-β.[23] Blocking these signaling pathways using inhibitors may be a potential strategy for BCSC-targeted therapies.

Wnt/β-Catenin Pathway

The Wnt/β-catenin signaling pathway regulates many physiological processes, such as self-renewal, growth, and regeneration of cells.[62] After a Wnt ligand binds to a Wnt receptor, such as heterodimeric frizzled-7 (Fzd7) or low-density lipoprotein receptor-related protein 6 (LRP6), the Wnt pathway is activated and the signal is transferred to β-catenin through several downstream processes. Upon activation, the dephosphorylated β-catenin enters the nucleus to activate Wnt target genes.[63] This signaling pathway is upregulated in many cancers, including breast cancer, and is considered to be a key factor in the maintenance and self-renewal of BCSCs. Thus, selective targeting of Wnt/β-catenin signaling may be a strategy to eliminate BCSCs and reduce breast cancer aggressiveness. For instance, Jang et al[7] demonstrated that Wnt/β-catenin signaling is relatively more active in BCSCs than in bulk tumor cells, which results in the therapeutic resistance of BCSCs. These investigators also designed CWP232228 (a small-molecule inhibitor) to antagonize β-catenin that binds to T-cell factor in the nucleus. Their results showed that CWP232228 can inhibit the growth of both BCSCs and bulk tumor cells. However, an interesting observation was that the BCSCs exhibited decreased growth than the bulk tumor cells, indicating that CWP23228 has some degree of selectivity toward BCSCs. In addition to small-molecule inhibitors, macromolecular agents such as antibodies have been reported to block the Wnt/β-catenin pathway. For example, Gurney et al[64] reported that vantictumab (a monoclonal antibody) inhibits tumor growth and reduces tumor-initiating cell frequency by interacting with five Fzd receptors to block canonical Wnt signaling.

Notch Signaling Pathway

An activated pathway in breast cancer cells namely, Notch signaling, plays a vital role in stem cell retention and differentiation and has attracted much attention in recent

times, as a target to inhibit breast cancer relapse and metastasis by eradicating BCSCs.[48,65] The Notch signaling pathway is activated by ligands (eg, Delta-like [DLL] 1, 3, and 4; and Jagged [JAG]1 and 2) that binding to Notch receptors (Notch1-4).[66,67] This binding results in cleavage of the Notch receptor by the enzyme γ-secretase to release the Notch intracellular domain (NICD). The released NICD translocates to the nucleus to activate Notch target genes. Therefore, inhibitors of γ-secretase and antagonists of Notch receptors or ligands have the potential to inhibit Notch activity in BCSCs. For example, Grudzien et al[67] reported that BCSCs possess greater Notch signaling than bulk tumor cells, and they demonstrated a reduction in sphere formation, proliferation, and/or colony formation in soft agar by blocking Notch signaling using pharmacological and genomic approaches (eg, by using MRK003, a γ-secretase inhibitor). Moreover, McClements et al[68] reported that specific peptides (ALM201 and AD-01) inhibited BCSCs in both ER^+ and ER^- breast cancer by downregulating DLL4 and Notch 4;[68] this study was the first to demonstrate the preclinical systemic activity of ALM201 and AD-01on breast cancer.

Hh Signaling Pathway

The Hh signaling pathway essentially regulates the maintenance, self-renewal, survival, and proliferation of BCSCs.[12,69,70] In this pathway, the binding of Hh ligand to the patched receptor of a neighboring cell reduces the inhibition of the transmembrane receptor protein Smoothened (Smo). Smo activation then leads to the release of the glioma-associated oncogene (Gli) family of transcription factors (Gli1/2/3), which undergo nuclear translocation to regulate the expression of Hh target genes. The overexpression of both Smo and Gli has been found in the BCSC subpopulation, therefore, Smo and Gli are potential targets for inhibiting the Hh signaling pathway to eliminate BCSCs.[12,71] In this regard, GANT61, a Gli1/2 inhibitor, was reported to decrease the percentage of cancer stem cells and enhance the anti-mitogenic activity of paclitaxel in several triple-negative breast cancer (TNBC) cell lines.[72] This result implicated that GANT61 as a potential therapeutic agent in TNBC. In addition, cyclopamine, the first Hh inhibitor to be identified, reduced the growth of breast cancer cells by binding to and inactivating Smo to suppress Gli1 expression.[73] These results demonstrate that therapeutic agents with the ability to inhibit Smo and Gli exhibit the potential to reduce the percentage of BCSCs in breast tumor tissue.

Hippo Signaling Pathway

Hippo signaling is regulated by a network of core kinase cascades and is a key regulator of tumorigenesis and stem cell renewal.[74] It was demonstrated that the dephosphorylation of yes-associated protein 1 (YAP1) and transcriptional co-activator with PDZ-binding motif (TAZ) results in their nuclear translocation, leading to the activation of Hippo target gene transcription.[74] YAP1 and TAZ are reported to be overexpressed in BCSCs of metastatic breast cancer, and thus, could be potential targets for inhibiting the Hippo signaling pathway and reduce the percentage of BCSCs.[75] Interestingly, Li et al[76] reported that inhibiting the transcriptional activities of YAP1 and TAZ with apigenin, a naturally occurring compound, reduced the stemness of TNBC cells. This indicates that apigenin is a promising therapeutic agent for the treatment of TNBC patients showing high YAP/TAZ activity.

TGF-β Signaling Pathway

TGF-β is the prototype of the TGF-β family of growth and differentiation factors. TGF-β can facilitate the transformation of cancer cells into cancer stem cells by the activation of epithelial–mesenchymal transition (EMT)–inducing transcription factors, resulting in drug resistance.[77–80] Li et al[78] showed that the pleiotropic effects of TGF-β influence chemotherapeutic drug resistance by modulating EMT, stemness, and apoptosis. TGF-β signaling has also been reported to play a vital role in the maintenance and functioning of BCSCs.[62,81] Therefore, targeting TGF-β signaling may be an effective strategy to treat breast cancer by inhibiting BCSCs. It was demonstrated by Liu et al[81] that Gd–metallofullerenol-based nanomaterial could eradicate BCSCs by inhibiting TGF-β signaling under normoxic conditions and suppressing both hypoxia-inducible factor (HIF)-1α and TGF-β activities under hypoxic conditions. An even more exciting fact is that the metallofullerenol nanomaterial Gd@C82(OH)22 is essentially nontoxic to normal mammary epithelial cells and can inhibit breast tumor initiation and metastasis by eliminating BCSCs.

Inducing BCSC Differentiation

Differentiation was demonstrated by Warrel et al[82] as an effective method of treating acute promyelocytic leukemia. In this study, after the patients were treated with all-trans retinoic acid (ATRA), it was found that their leukemic

promyelocytes failed to differentiate into mature granulocytes, indicating that preventing cancer stem cell differentiation maybe useful in the treatment of other cancers. Differentiation therapy targets cancer stem cells and alters their stemness to reduce the therapeutic resistance of cancer. Sun et al[31] demonstrated that a drug delivery system encapsulating ATRA and DOX effectively suppressed breast cancer by inducing BCSCs to differentiate into non-BCSCs, reducing their tumor initiation abilities and enhancing their sensitivity to DOX under the effect of ATRA. Pham et al[51] showed that the knockdown of CD44 with shRNA using lentivirus particles differentiated BCSCs into non-BCSCs, increasing their susceptibility to both chemotherapy and radiation. This indicated that CD44 knockdown is an effective strategy to eliminate the stemness of BCSCs and can be a potential strategy to treat breast cancer by targeting these cells.

Targeting Metabolism in BCSCs

Cancer stem cells exhibit specific metabolic properties, such as the metabolism of glucose and mevalonate.[32,52,70] For example, hexokinase 2 (HK2), a very important kinase involved in glucose metabolism, is overexpressed in BCSCs.[33,70] Thus, inhibiting HK2 is a potential method to eradicate BCSCs. In studies conducted previously, metformin (MET) has displayed anti-BCSC activity and enhanced the efficacy of chemotherapy in breast cancer by inhibiting HK2.[53] Moreover, inhibiting the mevalonate metabolic pathway with hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase blockers apparently suppressed the growth of BCSCs.[32] As another example, SAL was reported to reduce the stemness of BCSCs by inhibiting the Wnt pathway, which is a known regulator of cell metabolism.[83] It has also been reported that iron metabolism plays an essential role in cancer stem cells; therefore targeting iron metabolism may improve the therapeutic effect against many cancers, including breast cancer.[54] These examples demonstrate that the characteristic metabolic properties of BCSCs could be used as targets to treat them.

Targeting the Tumor Microenvironment

The microenvironment in which BCSCs are located plays an essential role in maintaining the functions of these cells.[24,84] This specific microenvironment is regulated by many factors such as mesenchymal stem cells (MSCs), immune cells, cancer-associated fibroblasts (CAFs), autocrine signals, the extracellular matrix and vascular network, oxygen pressure, and nutrient levels. In addition, this microenvironment can generate BCSCs by inducing the characteristics of cancer stem cells in non-BCSC.[55] It was reported that MSCs in breast tissue could expand from bone marrow-derived MSCs to regulate BCSCs by cytokine loops involving interleukin (IL)-6 and C-X-C motif ligand 7 (CXCL7) to expedite the growth of breast cancer.[85] CAFs were found to produce high levels of chemokine (C-C motif) ligand 2 (CCL2) to stimulate stem cell-specific features in breast cancer cells,[25] and CAFs were also found to regulate BCSCs via IL-6 and IL-8.[26] Tumor-associated macrophages (TAMs), a type of immune cell, were reported to facilitate the generation of cancer stem cells by secreting tumor necrosis factor-α (TNF-α) and TGF-β,[56] and promote BCSC phenotypes in murine breast cancer cells by affecting the epidermal growth factor receptor (EGFR)/signal transducer and activator of transcription 3 (STAT3)/sex-determining region Y-box 2 (SOX2) signaling pathway.[86] Mammary adipose tissues secrete adipokines to augment the features and proliferation of BCSCs.[87] Thus, factors affecting the BCSC microenvironment are potential targets for eliminating BCSCs to reduce the relapse, therapeutic resistance, and metastasis of breast cancer.

Agents Against BCSCs

Considering the effects of BCSCs on the initiation, maintenance, development, relapse, therapeutic resistance, and metastasis of breast cancer, many agents have been proposed to eliminate BCSCs. These agents are divided into three categories according to their physicochemical properties: small-molecule inhibitors/drugs, nucleic acid drugs, and protein drugs (Table 1).

Small-Molecule Inhibitors/Drugs

Small-molecule inhibitors/drugs account for the majority of current anti-BCSC agents. Quercetin suppressed the proliferation, self-renewal, and invasiveness of BCSCs in MDA-MB-231 cells by downregulating the expression of ALDH1 family, member A1 (ALDH1A1), C-X-C chemokine receptor type 4 (CXCR4), mucin 1 (MUC1), and EpCAM.[34] Sulforaphane inhibited BCSCs both in vitro and in vivo, as demonstrated by a mammosphere formation assay, ALDEFLUOR assay, and secondary tumor growth in mice; one potential mechanism is by downregulation of the Wnt/β-catenin pathway in BCSCs.[35] It was reported that curcumin inhibited BCSCs by suppressing both the sonic hedgehog

Table 1 Agents Against BCSCs

Type	Agents	Mechanism of Action	Status	References
Small-molecular inhibitors/drugs	Lovastatin	Inhibit SOX2 promoter transactivation	In vitro	[88]
	Quercetin	Low the expression of ALDH1A1, CXCR4, MUC1 and EpCAM	In vitro	[34,89]
	Curcumin	Suppress SHH and Wnt/β-catenin pathways	In vitro	[36]
	Tocopherols	Estrogen-dependent and Oct-4 mediated mechanisms	In vitro	[90]
	Valproic acid	An inhibitor of histone deacetylases	In vitro	[91]
	GANT61	A Hh inhibitor	In vitro	[20]
	Actinomycin D	Down-regulation of Sox-2	In vitro	[92]
	Forskolin	Activation of protein kinase A system (PKA) leads to mesenchymal-to-epithelial transition and loss of tumor-initiating ability	In vitro	[93]
	S2E	Glutathione S-transferase omega 1 inhibitors	In vitro	[94]
	Niclosamide	Targets NF-κB, Wnt/β-catenin and Notch pathway of BCSCs	In vivo	[95]
	Simvastatin	Inhibition of mevalonate metabolism	In vivo	[32]
	Sulforaphane	Downregulate the Wnt/β-catenin pathway	In vivo	[35]
	Cyclopamine	Inhibitor of the Hh pathway	In vivo	[96]
	Iadademstat	Inhibitor of the lysine-specific demethylase 1	In vivo	[97]
	KU758, KU711	C-terminal heat shock protein 90 inhibitors	In vivo	[98]
	Dasatinib	A Src kinase family inhibitor	In vivo	[5]
	BKM120	Inhibit the PI3K/Akt signaling pathway in SCs	In vivo	[99]
	Pyrvinium pamoate	WNT pathway inhibitor	In vivo	[100]
	Mifepristone	By down-regulating kruppel-like factor 5 expression	In vivo	[101]
	Chloroquine	Deregulation of janus kinase 2 and DNA methyltransferase 1	In vivo	[102]
	Kazinol-E	Inhibitor of extracellular regulated protein kinases	In vitro	[103]
	Salinomycin	Result in the loss of expression of BCSC genes	In vivo	[37]
	CWP232228	Inhibiting β-catenin-mediated transcription	In vivo	[7]
	Apigenin	Hippo pathway inhibitor	In vivo	[76]
	Metformin	Inhibit cellular transformation	In vivo	[104]
	Thioridazine	Targets dopamine receptor of BCSCs	In vivo	[105]
	Vitamin D compounds (1α, 25 (OH) 2D3, BXL0124)	Noth signaling pathway inhibitor	In vivo	[106]
	Doxycycline	Inhibitor of mitochondrial biogenesis	Clinical pilot study	[107]

(*Continued*)

Table 1 (Continued).

Type	Agents	Mechanism of Action	Status	References
Nucleic acid	miRNA-200c	Reduce BCSCs by targeting B cell-specific moloney murine leukemia virus integration site1(BMI1)	In vitro	[108]
	miR-141	Reduce BCSCs by targeting signal transducer and activator of transcription 5 a (Stat5a)	In vitro	[109]
	miR-34c	Inhibit BCSCs by targeting Notch4	In vitro	[110]
	miR-128	Inhibit BCSCs by targeting BMI1 and ATP Binding Cassette Transporter C5 (ABCC5)	In vitro	[111]
	miR-140	Inhibit BCSCs by targeting ALDH1 and SOX9	In vitro	[112]
	NF-κB shRNA	Decrease mammosphere and colony formation and lower $ALDH^+$ CSCs population	In vitro	[38]
	Let-7	Reduce BCSCs by targeting High mobility group AT-hook 2(HMGA2)	In vivo	[113]
	miRNA-100	Attenuate expression of the CSCs regulatory genes SMARCA5, SMARCD1, and BMPR2.	In vivo	[6]
	miR-200b	Inhibit BCSCs by targeting SUZ12, H3K27me3 of E-cadherin and other genes	In vivo	[114]
	miR-30	Inhibit BCSCs by targeting Ubc9 and ITGB3	In vivo	[115]
	miR-27a	Inhibit BCSCs by targeting ZBTB10	In vivo	[116]
	miR-27b	Inhibit BCSCs by targeting ENPP1	In vivo	[117]
	miR-7	Inhibit BCSCs by targeting KLF4, SETDB1	In vivo	[118]
	miR-34a	Reduce BCSCs by targeting Notch1	In vivo	[119]
	integrin α9 gene	Knockout of integrin α9 using CRISPR/Cas9 technology reduced TNBC cell cancer stem cell (CSC)-like property	In vivo	[120]
	YB-1 gene	Knockout of YB-1gene using CRISPR/Cas9 technology inhibited the proliferation of BCSCs	In vivo	[121]
Protein	Cholera toxin	Activation of PKA leads to mesenchymal-to-epithelial transition and loss of tumor-initiating ability	In vitro	[93]
	Trastuzumab	Inhibit the HER-2 related BCSC-activating pathways	In vivo	[122]
	PF-06647020	Inhibit Wnt signaling pathway	In vivo	[123]
	OMP-18R5	Inhibit Wnt signaling pathway	In vivo	[64]
	Cirmtuzumab	Reduce activation of Rho-GTPases, Hippo-YAP/TAZ, or BMI1	In vivo	[124]
	Anti-CDH11 antibody	Inhibit epithelial-to-mesenchymal transition and repressed cancer stem cell-like phenotype	In vivo	[18]
	TmSm(T34A)	Down-regulating the expression of Cyclin D1	In vivo	[125]
	P245	Against the CD44 cell surface receptor	In vivo	[57]
	RO5429083/RG7356	Modifications of the mitogen-activated protein kinase pathway	In vivo	[126]
	B6H12.2	Blockade the function of CD47	In vivo	[127]
	dCD133KDEL	Inhibition of CD133	In vivo	[59]

(SHH) and Wnt/β-catenin pathways, which play essential roles in maintaining the stemness of BCSCs.[36] A noncanonical Hh inhibitor, GANT61, was reported to downregulate the expression of GLi1, GLi2, and/or SHH to decrease the levels of BCSCs induced by estradiol in ER-positive breast cancer cells.[20]

Vitamin D compounds (1α,25[OH]2D3; BXL0124) were found to effectively reduce BCSCs in TNBC by repressing Notch1, Notch2, Notch3, JAG1, and JAG2 to inhibit the Notch signaling pathway in BCSCs[106] Apigenin suppresses the stemness of BCSCs by targeting YAP and TAZ, two main downstream effectors of the Hippo pathway. This inhibits YAP/TAZ-TEAD complex activity, which is essential for tumor initiation and maintenance of the self-renewal ability of BCSCs.[76] Simvastatin, an inhibitor of HMG-CoA reductase, blocks the biosynthesis of mevalonic acid to reduce BCSCs both in vitro and in vivo.[32]

Chloroquine, an autophagic inhibitor, reduces BCSCs in TNBC by inhibiting the Janus-activated kinase 2 (JAK2)-STAT3 signaling pathway, which sensitizes TNBC stem cells to paclitaxel by inhibiting autophagy.[102] By activating protein kinase A, forskolin induces EMT in mammary epithelial cells, which causes them to lose the ability to initiate tumors and sensitizes them (in vitro) to conventional chemotherapeutic agents, such as DOX.[93] SAL was shown to inhibit mammary tumor growth and reduce the proportion of BCSCs, and its effect was more than 100 times that of paclitaxel.[37] A clinical pilot study by Scatena et al[107] found that doxycycline, an Food and Drug Administration (FDA)-approved antibiotic, could reduce BCSC markers (CD44 and ALAH1) and eliminate BCSCs in breast cancer patients by inhibiting the mitochondrial-related proteins that were overexpressed in many cancer stem cells, including BCSCs. However, the authors emphasized that additional clinical studies with larger numbers of patients will be needed to validate this promising pilot study. In general, there have been many reports about small-molecule agents with anti-BCSC capabilities, but most of these studies were in the preclinical stages. More research is needed to confirm the anti-BCSC effects of these agents and reduce the toxicities induced by their nonselective distribution in vivo.

Nucleic Acid Drugs

Gene therapy is a potential method to target BCSCs. In theory, downregulation of BCSC surface markers or blockade of signaling pathways using siRNA, shRNA, or miRNA agents has the potential to suppress the function of BCSCs. For example, NF-κB shRNA was reported to decrease the percentage of $ALDH^+$ BCSCs and mammosphere colony formations.[38] Hu et al[39] found that Oct-4 gene suppression by Otc-4 siRNA induced BCSC apoptosis via inhibition of the Oct-4/Tcl1/Akt1 signaling pathway. miRNAs, post-transcriptional regulators of various cellular functions, have also been reported as potential anticancer agents. Elevating the expression of miR-100 was shown to decrease the production of BCSCs by attenuating the expression of the cancer stem cell regulatory gene (SWI/SNF-Related Matrix-Associated Actin-Dependent Regulator of Chromatin Subfamily D Member 1, SMARCD1) and bone morphogenetic protein receptor type 2 (BMPR2).[6] miR-34a, a tumor-suppressor miRNA, has the capacity to affect the properties of BCSCs and enhance their susceptibility to doxorubicin treatment by targeting Notch1.[119]

In addition to siRNA, shRNA, and miRNA, gene editing technologies, especially the CRISPR/Cas9 genome-editing system, have generated enormous interest in the field of gene therapy.[128,129] This technology provides a robust tool to generate knockout cells or animal models quickly, exhibiting great potential for applications in the treatment of cancer.[130] For instance, Wang et al[120] knocked out integrin α 9 (ITGA9) in TNBC cells using CRISPR/Cas9 technology and found that ITGA9 knockout noticeably attenuated the properties of BCSCs in TNBC cells and the angiogenesis, growth, and metastasis of tumors by promoting β-catenin degradation. Yang et al[121] using CRISPR/Cas9 to knock out the Y-box binding protein 1 (YB-1) gene, found that YB-1 deletion inhibited the proliferation of BCSCs, leading to cell cycle arrest and apoptosis, and induced irreversible differentiation of cancer stem cells. This indicates that YB-1 plays an important role in maintaining the stemness of BCSCs and reverting the differentiated tumor cells back to cancer stem cells.

Protein Drugs

Protein drugs, especially antibodies, are being explored to suppress BCSCs by targeting their surface markers or various signaling pathways. For instance, OMP-185R, a monoclonal antibody, suppressed canonical Wnt signaling by blocking the Fzd receptor family to reduce the growth of many tumors (including breast cancer tumors) and the frequency of tumor-initiating cells.[64] P245 mAb, which targets CD44, reduced the growth and prevented

recurrence of tumors in human breast cancer xenografts. These effects were believed to be attributed to the induction of antiproliferative cytokines.[57] The recombinant protein TmSm (T34A) was demonstrated to prevent the proliferation and growth of BCSCs by downregulating the expression of cyclin D1 and significantly inducing the apoptosis of BCSCs.[125]

Problems of Current BCSC-Specific Agents

Although obvious progress has been achieved with BCSC-specific agents, some serious challenges still remain. First, similar to conventional chemotherapeutic drugs, BCSC-specific agents may possess characteristics that are undesirable in vivo. For instance, accumulating evidence has demonstrated that miRNA, siRNA, and shRNA have great potential to be used as anti-BCSC agents; however, RNA-based therapies are limited by many obstacles in vivo including their degradation in blood, poor cellular uptake, and potential systemic toxicity, the latter of which results from poor tissue targeting.[38,132,133] The clinical applications of certain anti-BCSC agents, such as curcumin and quercetin, have been limited because of their poor absorption and rapid metabolism.[34,41,89,134] Second, BCSCs share many characteristics (such as self-renewal and quiescence) with normal stem cells. Moreover, none of the presently reported anti-BCSC agents can distinguish BCSCs from normal stem cells; therefore, these agents are potentially toxic to normal stem cells. For instance, although most γ-secretase inhibitors show anti-BCSC properties, they may concomitantly damage normal stem cells.[12,60,63,135,136]

NDDSs Against BCSCs

There is an urgent need to solve the problems of current anti-BCSC agents, such as poor solubility, instability, unfavorable biodistribution, and high toxicity induced by off-target effects.[3,130,137] NDDSs have the potential to address this need. NDDSs can passively target tumor tissues owing to their enhanced permeability and retention (EPR) effects. Moreover, the BCSC-targeted effects of NDDSs can be further enhanced by their surface modification with suitable ligands that interact with overexpressed receptors on the surface of BCSCs. A deeper understanding of the biology of BCSCs and numerous advances in nanotechnology have resulted in increasing numbers of NDDSs being developed to treat breast cancer by eliminating BCSCs. The delivery strategies of NDDSs against BCSCs mainly include: delivery of anti-BCSC agents to tumors; combinational delivery of chemotherapeutics and anti-BCSC agents to tumors; active-targeted delivery of anti-BCSC agents and/or chemotherapeutics agents to tumors. In this section, NDDS-targeted BCSCs are summarized and categorized in Tables 2–4, according to their cargo delivery and modifications.

Delivery of Chemotherapeutic Agents to BCSCs

Most properties of the currently reported BCSC-specific chemotherapeutic agents are undesirable in vivo and are similar to those of traditional chemotherapeutic agents. One application of NDDSs is to qualify therapeutic agents and drug candidates; Table 2 displays an overview of the NDDSs that have been used to deliver BCSC-specific chemotherapeutic agents. For example, curcumin, a polyphenol derived from the ancient Asian spice turmeric, has been reported to target cancer stem cells by downregulating signaling pathways such as Wnt, Notch1, and NF-κB, and reducing the expression of ALDH, a marker of cancer stem cells.[171–173] However, its clinical development has been restricted by its hydrophobicity, poor in vivo stability, and rapid metabolism. Gülçür et al[41] developed a novel nanomicellar formulation of curcumin to overcome these shortcomings. Encapsulating curcumin in sterically stabilized micelles (C-SSM) significantly enhanced its aqueous solubility and stability. Furthermore, curcumin-encapsulated C-SSM clearly enhanced the efficacy of curcumin against both breast cancer cells and BCSCs.

Wedelolactone, an active polyphenolic compound of *Sphagneticola calendulacea* and *Eclipta prostrata*,[174] was demonstrated to kill many cancer cells—including breast cancer cells—but its disadvantages, such as poor solubility and bioavailability, restrict its clinical application.[40,175] Das et al[40] formulated wedelolactone-encapsulated poly (lactic-co-glycolic acid) (PLGA) nanoparticles (nWdl) to target BCSCs and overcome their shortcomings. nWdl was shown to sensitize BCSCs to the effects of wedelolactone by downregulating SOX2 and adenosine-triphosphate (ATP)-binding cassette super-family G member 2 (ABCG2).

Table 2 Delivery of BCSC-Specific Small Molecular Agents

Ligand/Receptor	Therapeutic Agent	Drug Delivery System	Status	References
	Chloroquine	Triphenylphosphonium-functionalized hyperbranched polymer nanocarrier	In vitro	[138]
	Salinomycin	Gold nanoparticles (AuNPs) coated with poly(ethylene glycol)	In vitro	[139]
	Doxorubicin	DOX-Hyd@AuNPs nanoparticles	In vivo	[13]
	CRLX101	Camptothecin-containing nanoparticle-drug conjugate	In vivo	[140]
	Wedelolactone	PLGA nanoparticle	In vivo	[40]
	Zileuton™	Pluronic® F127 polymer micelles	In vivo	[141]
	Cyclopamine	Liquid–lipid nanoparticles	In vivo	[96]
	Doxorubicin	Pluronic polymeric micelles	In vivo	[142]
	All-trans retinoic acid	Stealth liposomes	In vivo	[143]
Vasoactive intestinal peptide/VIP receptors	Curcumin	DSPE-PEG2000 nanomicelles	In vitro	[41]
Ferritin/transferritin receptor 1	Mertansine	Biomimetic nanocages of apoferritin	In vitro	[144]
Chitosan/CD44	Doxorubicin	Pluronic F127 cross-linked and surface-decorated with chitosan nanoparticles	In vivo	[145]
Glucose/glucose transporter 1	γ-secretase inhibitors	MSN-PEI-Gluc Nanoparticle	In vivo	[146]
HA/CD44; DCLK1 monoclonal antibody/DCLK1	Doxorubicin	HA and DCLK1monoclonal antibody modified PLGA nanoparticles	In vivo	[147]
Anti-CD133;TAT peptides/CD33	Tirapazamine	Mesoporous silica nanoparticle (MSN)	In vivo	[148]
RGD/integrin alpha5 (ITGA5)	Diacidic norcantharidin	Lipid-polymer hybrid (LPH) nanoparticle	In vivo	[46]
CD44 antibody/CD44	Hsp90 inhibitor	CD44-Fe3O4@SiNPs	In vivo	[149]
F3 peptide/nucleolin	Doxorubicin	Liposomes functionalized with the nucleolin-binding F3 peptide	In vivo	[150]
EpCAM aptamer/EpCAM protein	Aspirin	Nanoexosomes	In vivo	[45]
Herceptin/Her-2	Metformin	Herceptin-Conjugated Liposome	In vivo	[42]

SAL, a polyether ionophore antibiotic, has demonstrated great potential in eliminating BCSCs, but its clinical application is hindered by its poor aqueous solubility and severe systemic toxicity.[83,139,176,177] Considering the need for an efficient drug while reducing potential damage to normal tissues, Zhao et al[139] developed biocompatible gold nanoparticles coated with poly(ethylene glycol) (PEG) that were conjugated with SAL. These SAL-loaded gold nanoparticles enhanced the therapeutic efficacy of SAL against BCSCs derived from a $CD24^{low}/CD44^{high}$ subpopulation.

MET, an anti-type 2 diabetic drug, was reported to affect breast cancer at low dosages by targeting BCSCs; however, its anti-breast cancer efficacy is hindered by its low bioavailability and nonselective biodistribution. Lee et al[42] demonstrated that MET-encapsulated trastuzumab-conjugated immunoliposomes (Her-LP-MET) could target BCSCs and inhibit both their proliferation and migration. The combination of Her-LP-

Table 3 Delivery of BCSC-Specific Nucleic Acid Drugs

Ligand/Receptor	Therapeutic Agent	Drug Delivery System	Status	References
	NF-κB shRNA	Carbamate-mannose modified PEI	In vitro	[38]
	miR-34a	hTERT promoter-driven VISA nanoparticle	In vivo	[151]
	AKT2 siRNA	Pluronic® F127 micelles with polyplexes	In vivo	[152]
Anti-HER2 nanobody/ HER2	Apoptosis-inducing tBid gene	Anti-HER2 nanobody (Nb)-conjugated polyamidoamine (PAMAM) polyplexes	In vitro	[153]
APTEDB/EDB-FN	EDBsiRNA	Liposomal system (APTEDB-LS-siRNAEDB)	In vivo	[154]
Glucose/glucose transporter 1	Polo-like kinase 1 (PLK1) siRNA	Glucose-installed targeted nanoparticles	In vivo	[155]

MET with free DOX resulted in better breast tumor remission than treatment with only free DOX.

It was suggested by Sun et al[13] that rationally designed drug delivery systems could significantly enhance the cancer stem cell therapy of conventional chemotherapeutic drugs such as DOX by delivering these drugs into cancer stem cells. They formulated DOX-tethered gold nanoparticles (DOX-Hyd@AuNPs) and demonstrated that DOX-Hyd@AuNPs could inhibit the growth of breast cancer without increasing the BCSC subpopulation in the tumor by delivering more DOX into the BCSCs. This process overcame the intrinsic resistance of BCSCs arising from P-glycoprotein (P-gp) drug efflux.

Delivery of Nucleic Acid Therapeutics to BCSCs

In addition to increasing the solubilization of low-solubility drugs, NDDSs have the capacity to enhance the stability and cellular uptake of macromolecules such as siRNA, shRNA, and miRNA which could potentially treat cancer.[130,178] AKT2, a major downstream effector of the phosphatidylinositol 3-kinase (PI3K) pathway, was reported to be associated with cancer stem cell tumorigenicity and the malignant phenotype of cancer cells.[179–181] The silencing of AKT2 with siRNA has the potential to inhibit the development and recurrence of tumors. Nevertheless, the rapid degradation and poor cellular uptake of siRNA are challenges for siRNA-based therapies. Using NDDSs to deliver siRNA may be a promising strategy to increase the stability and cellular delivery of siRNA. Rafael et al[152] developed an innovative nanocarrier system composed of Pluronic® F127-based micelles associated with polyethylenimine (PEI)-based polyplexes to deliver AKT2 siRNA. This AKT2-siRNA delivery system displayed strong suppressive effects on BCSCs migration and invasion in breast cancer.

NF-κB plays an important role in maintaining the properties of BCSCs in various types of breast cancer.[182,183] Therefore, it is possible to target BCSCs by downregulating the expression of NF-κB proteins using RNA interference, including siRNA and shRNA. Compared to siRNA, shRNA is more stable; it is a double-stranded RNA molecule with a tight hairpin structure.[184] Ke et al[38] synthesized a carbamate-mannose-modified PEI (CMP) for the targeted delivery of NF-κB shRNA to BCSCs. These CMP/NF-κB-targeted shRNA nanocomplexes were demonstrated to reduce the percentage of BCSCs, inhibit the formation of mammospheres and colonies, suppress the migration and invasion of breast cancer cells, and sensitize breast cancer cells to treatment with doxorubicin-loaded micellar nanoparticles.

miRNAs are essential post-transcriptional regulators of many cellular functions. miR-34a is a tumor-suppressor miRNA that has been reported to have the capacity to attenuate the properties of BCSCs.[119] Lin et al[151] established a human telomerase reverse transcriptase (hTERT) promoter-driven VP16-Gal4-WPRE integrated systemic amplifier (VISA) delivery system for miR-34a (TV-miR-34a) plasmid. They demonstrated that TV-miR-34a clearly eliminated BCSCs both in vitro and in vivo in a safe and efficient way and showed increased therapeutic efficacy toward breast cancer cells in combination with docetaxel. Further mechanistic studies revealed that TV-miR-34a attenuated BCSC properties, promoted adherence, and boosted the differentiation of BCSCs by directly targeting chromosome 22 open reading frame 28 (C22ORF28).

Table 4 Combinational Delivery of Chemotherapeutics and CSC-Specific Agents

Ligand/Receptor	Therapeutic Agent	Drug Delivery System	Status	References
	GANT61+Curcumin	PLGA NPs	In vitro	[156]
	Camptothecin+GRP78 siRNA/CLUsiRNA	DOTAP liposomes	In vitro	[157]
	miRNA-200c+Paclitaxel	Solid lipid nanoparticles	In vitro	[158]
	Salinomycin+Doxorubicin	Cross-linked multilamellar liposomal	In vivo	[159]
	All-trans-retinoic acid +Doxorubicin	Poly(ethylene glycol)-block-polylactide (PEG-b-PLA)	In vivo	[31]
	Staurosporine+Epirubicin	PEG-b-poly(aspartate-hydrazide-epirubicin) copolymer	In vivo	[160]
	siBMI-1+Docetaxel	Ationic-lipid-assisted nanoparticles PEG5Kb-PLGA12K;cationic lipid BHEM-Chol	In vivo	[17]
	Doxorubicin+SN38	PEG-CH=N-DOX prodrug	In vivo	[43]
	Curcumin+Doxorubicin	mPEG-PLGA-Pglu nanoparticle	In vivo	[15]
	Docetaxel+Salinomycin	PLGA/TPGS nanoparticle	In vivo	[161]
	Doxorubicin+Hymoquinone	Cockle shell-derived aragonite CaCO3 nanoparticles	In vivo	[162]
	Paclitaxel+Thioridazine +HY19991	Enzyme/pH dual-sensitive nanoparticle with a micelle-liposome double-layer structure	In vivo	[163]
HA/CD44	Salinomycin+Paclitaxel	PLGA nanoparticle	In vitro	[164]
HA/CD44	Doxorubicin+ tariquidar	dendritic polyglycerol-conjugated, mesoporous silica-based targeting nanocarriers	In vitro	[165]
oHA/CD44	Curcumin+Paclitaxel	Double pH-sensitive nano-eggs	In vivo	[166]
HA/CD44	Doxorubicin+irinotecan	Hyaluronic acid-decorated dual responsive nanoparticles	In vivo	[167]
CD44 antibody/CD44	Paclitaxel+Salinomycin	Single-walled carbon nanotubes	In vivo	[44]
HA/CD44	Doxorubicin+ICG	HA-hyaluronan-decorated fullerene-silica	In vivo	[168]
HA/CD44	Paclitaxel+Curcumin	HA-HAD-PLGA nanoparticles	In vivo	[169]
Ferritin/Transferritin receptor 1	Epirubicin+DIR	Biomimetic nanocages of apoferritin	In vivo	[47]
CD44 aptamer TA6/ CD44	Doxorubicin+AKT inhibitor peptide	Aptamer-conjugated DNA nanotrain TA6NT-AKTin-DOX	In vivo	[16]
HA/CD44	Doxorubicin+Cyclopamine	Hyaluronic acid functional amphipathic and redox-responsive polymer particles	In vivo	[170]

Combinational Delivery of Chemotherapeutics and Cancer Stem Cell-Specific Agents

An increasing amount of evidence indicates that tumors are heterogeneous tissues with different types of cells, such as cancer stem cells and non-cancer stem cells.[137] Some therapeutic agents have been reported to eliminate cancer stem cells; however, cancer cells can also spontaneously transition to cancer stem cells; thus, the depletion of cancer stem cells alone is neither sufficient nor effective as a therapeutic measure.[3,130,185] Instead of targeting only cancer stem cells or non-cancer stem cells, combination strategies designed to simultaneously eradicate both cell

types may have the potential to improve therapeutic outcomes. Table 4 shows representative reports about combination strategies that target both cancer and non-cancer stem cells.

To eradicate both breast cancer cells and BCSCs, Kim et al[159] developed cross-linked multilamellar liposomal vesicles (cMLVs) to co-deliver DOX (a conventional chemotherapeutic drug) and SAL (an inhibitor of BCSCs), producing cMLV(DOX+SAL) particles. The antitumor results of cMLV(DOX+SAL) in vitro and in vivo demonstrated that the co-delivery of DOX and SAL in a single cMLV greatly inhibited both breast cancer cells and BCSCs, which may be attributed to the simultaneous delivery of the two drugs to the tumor tissue by cMLV(DOX+SAL).[159] Similarly, Zhang et al[160] evaluated the therapeutic efficacy of micelles that were co-loaded with the cytotoxic drug epirubicin (EPI) and the BCSC inhibitor staurosporine (STS) to treat breast cancers, especially when the tumors recurred after traditional chemotherapy. Theses results demonstrated that the STS/EPI-loaded micelles can potentially treat naïve orthotopic 4T1-luc breast tumors and their recurrent EPI-resistant counterparts by suppressing breast cancer cells together with the BCSC-associated subpopulation, such as the $ALDH^+$ and $CD44^+/CD24^-$ subpopulations.

NDDSs have co-delivered various traditional chemotherapeutic drugs with different antitumor mechanisms and exhibited the potential to eliminate both BCSCs and non-BCSCs. For instance, Sun et al[43] developed a cargo-free and pH-responsive nanomedicine for the co-delivery of a pH-responsive prodrug of DOX and 7-Ethyl-10-hydroxycamptothecin (SN38) to target breast cancer. The results showed that this nanomedicine significantly increased drug accumulation at the tumor site and simultaneously eradicated both BCSCs and non-BCSCs to achieve a superior antitumor efficacy in vivo. The excellent anti-BCSC capability of this developed nanomedicine may be attributed to the suppression of topoisomerase I (TOP I) and TOP II by DOX and SN38, respectively. Similarly, Wang et al[167] developed a nanoparticle loaded with DOX and irinotecan to inhibit both TOP I and TOP II, and the results showed that this co-delivery system noticeably enhanced the eradication of BCSCs with no evident systemic toxicity both in vitro and in vivo.

NDDSs can also be used for the co-delivery of traditional chemotherapeutic agents and nucleic acid agents such as siRNA, shRNA, and miRNA. For example, Chen et al[17] designed cationic-lipid-assisted nanoparticles to co-encapsulate docetaxel (DTXL, a traditional chemotherapeutic agent) and an siRNA targeting BMI-1 (siBMI-1, a nucleic acid agent) by the double emulsion method, producing $^{DTX}LNP_{siBMI-1}$ nanoparticles. These nanoparticles could effectively deliver therapeutic agents to both bulk cancer cells and BCSCs to produce combinational effects in the treatment of breast cancer. The bulk cancer cells were killed by DTXL and the expression of BMI-1 in the BCSCs was downregulated by siBMI-1, thereby eliminating them by enhancing their chemosensitivity to DTXL by reducing stemness. In the MDA-MB-231 xenograft model, $^{DTX}LNP_{siBMI-1}$ completely inhibited tumor growth and prevented recurrence, which was attributed to its capacity to kill both bulk cancer cells and BCSCs. Similarly, Samson et al[157] developed glucose-regulated protein 78 (GRP78)-targeted 1,2-dioleoyloxy-3-trimethylammoniumpropane (DOTAP) liposomes to deliver either camptothecin (CPT) and GRP78 siRNA (named DOTAP-CPT-siGRP78) or CPT and clusterin (CLU) siRNA (named DOTAP-CPT-siCLU). Both DOTAP-CPT-siGRP78 and DOTAP-CPT-siCLU exhibited stronger breast cancer cell- and BCSC-targeted activities than free CPT, confirming the synergistic effects of co-delivering anticancer drugs and siRNAs.

Additionally, NDDSs were explored to co-deliver protein agents targeting BCSCs and traditional chemotherapeutic agents to treat breast cancer. For instance, Xu et al[16] designed and prepared an aptamer-conjugated DNA nanotrain for the co-delivery of DOX and AKTin (an AKT inhibitor peptide); this drug delivery system was named TA6NT-AKTin-DOX. The efficacy of TA6NT-AKTin-DOX was evaluated on MCF-7 BCSCs and tumors generated by injecting BCSCs into nude mice. The results demonstrated that TA6NT-AKTin-DOX exhibited better efficacy than free DOX and various DNA nanotrains both in vitro and in vivo. The synergistic response of TA6NT-AKTin-DOX may be explained by AKTin, which can overcome the drug resistance of BCSCs via inhibition of the AKT signaling pathway.[16]

Moreover, multiple therapeutic agents with various anticancer mechanisms can be co-encapsulated in a single NDDSs with a high capacity to synergistically kill tumors. For example, paclitaxel (PTX, a chemotherapeutic agent), thioridazine (THZ, an anti-BCSC agent), and HY1991 (HY, a programmed cell death 1 [PD-1]/programmed cell death ligand 1 [PD-L1] inhibitor) were incorporated into an enzyme/pH dual-sensitive nanoparticle with a micelle-liposome double-layer structure. This PTX/THZ/HY-co-loaded drug delivery system displayed excellent anti-breast cancer efficacy and prolonged the lifespan of tumor-bearing

mice. This observation was attributed to the combination of chemotherapy, anti-BCSC therapy, and immune checkpoint blockade therapy.[163]

Active-Targeting NDDSs

The BCSC-targeting capacity of NDDSs can be further increased by surface modification of the NDDSs with suitable ligands that can interact with overexpressed cell-surface proteins on the BCSCs, such as CD44, EpCAM, integrin α5 (ITGA5), extra domain B of fibronectin (EDB-FN), transferrin receptor 1 (TfR1), and scavenger receptor class A membrane 5 (SCARA5). This should achieve active-targeting drug delivery, by enhancing the accumulation of drugs in targeted cells and reducing off-target effects.

For instance, Al Faraj et al[44] demonstrated that modification with CD44 antibody significantly enhanced SAL and PTX-conjugated SWCNT nanocarrier accumulation in both breast cancer cells and BCSCs in a xenograft murine model, providing a potential method for the effective treatment of breast cancer by targeting and eliminating both tumor cell and BCSC populations. Hyaluronic acid and chitosan were also reported to be CD44 ligands and could be used for the active targeting of CD44-overexpressing cells. For example, Yang et al[169] fabricated a hyaluronic acid lipoid by attaching a lipoid to the surface of PLGA nanoparticles to construct a vehicle for co-delivering PTX and curcumin to BCSCs. Owing to the interaction between hyaluronic acid of the fabricated lipoid and the cell-surface CD44 receptors on the BCSCs, it was shown that the BCSC-targeting ability of the fabricated hyaluronic acid lipoid were significantly enhanced, which allowed the fabricated lipoid to suppress the proliferation and migration of BCSCs. Moreover, the fabricated hyaluronic acid lipoid displayed excellent anticancer effects against MCF-7 xenograft tumor models by simultaneously suppressing the growth of breast cancer cells and BCSCs. Similarly, Rao et al[145] formulated DOX-loaded polymeric nanoparticles decorated with chitosan on their surface to target the overexpressed CD44 receptors on tumor reinitiating cancer stem-like cells. The nanoparticles enhanced six times of cytotoxicities compared with free doxorubicin for the eradication of $CD44^+$ cancer stem-like cells in 3D mammary tumor spheroids, and reduced tumor size with no obvious systemic toxicity in an orthotopic xenograft model by significantly increasing DOX accumulation in the tumors while reducing it in normal organs.

EpCAM is a surface marker on cancer stem cells that can be used to target them.[29,30] For instance, Tran et al[45] comprehensively studied the antitumor effects of nanoamorphous aspirin-loaded exosomes and showed that they had an unprecedented cancer stem cell eradication capacity. These authors also modified the exosomes with an aptamer specifically targeting EpCAM, which they found could further enhance the active-targeting ability of the exosomes.

The canonical Wnt/β-catenin pathway plays essential roles in the generation and maintenance of both cancer and normal stem cells. Thus, cancer therapy using inhibitors of the Wnt/β-catenin pathway may be toxic to normal stem cells. To achieve the specific inhibition of β-catenin in cancer cells, Li et al[46] proposed a strategy to suppress the stemness and metastasis of TNBC by developing an ITGA5-targeting lipid–polymer hybrid (LPH) nanoparticle modified with an ITGA5 ligand (a commercialized RGD motif, Arg-Gly-Asp) for the TNBC-targeted delivery of diacidic norcantharidin (NCTD). The developed RGD-decorated LPH nanoparticles significantly enhanced the accumulation of the delivered drug in orthotopic mammary TNBC tumors and lung metastatic tumors in nude mice. The nanoparticles also reduced the growth and metastasis of TNBC compared to that of free NCTD and non-modified LPH nanoparticles by downregulating β-catenin.

EDB-FN plays essential roles in the maintenance and growth of BCSCs, as well as in the expression of genes encoding surface markers on BCSCs and controlling their self-renewal.[154] Therefore, EDB-FN may be used as a biomarker for both targeting and treating BCSCs. APT_{EDB}, an EDB-FN-specific peptide, was used as a cancer-targeting ligand by Sun et al[154] to modify liposomes encapsulating EDB-FN siRNAs, forming a liposomal system (APT_{EDB}-LS-$siRNA^{EDB}$) with the potential to simultaneously target and knockdown EDB-FN in breast cancer treatment. It was found that APT_{EDB}-LS-$siRNA^{EDB}$ significantly increased the accumulation and cellular uptake of the delivered EDB-FN siRNA in EDB-FN-positive BCSCs in cultured cells and tissues compared to those in non-targeted liposomes. Moreover, APT_{EDB}-LS-$siRNA^{EDB}$ could knockdown EDB-FN both in vitro and in vivo, effectively treating EDB-FN-positive BCSC-derived tumors by eradicating the self-renewal ability of BCSCs.

Because of specific binding to the highly expressed TfR1 and SCARA5, ferritin is a promising nanoplatform for the

efficient targeting and deep penetration of tumors.[47] Recently, Tan et al[47] formulated ferritin nanocages loaded with 1.1-dioctadecyl-3,3,3,3-tetramethylindotricarbocyanine iodide (DBN) and EPI for the targeted treatment of breast cancer. Both DBN and EPI collected in large numbers at the tumor site, permeated throughout the tumor mass, and accessed the BCSCs in a metastatic 4T1-induced tumor model, which was attributed to the tumor-homing and biomimetic properties of the ferritin nanocages. It was noted that the DBN and EPI-loaded, BCSC-accessing nanocages considerably suppressed primary tumor growth with the notable elimination of BCSCs in the tumor mass, and significantly suppressed lung metastasis under the combined effect of photothermal and chemo therapies.

In addition to BCSC-specific surface proteins, some membrane proteins that are overexpressed on both BCSCs and non-BCSCs could be utilized to enhance the targeting ability of NDDSs. The identification of surface receptors overexpressed on both cancer and non-cancer stem cells is important for successful cancer treatment because the latter can transform into cancer stem cells via plastic EMT. For example, Fonseca et al[150] demonstrated that nucleolin receptors are overexpressed in both the BCSCs and non-BCSCs found in TNBC. These authors developed DOX and C6-ceramide co-loaded liposomes coated with nucleolin-binding F3 peptides to reduce the plasticity and adaptability associated with BCSCs and non-BCSCs. The developed F3 peptide-targeted liposomes exhibited significantly increased cellular toxicity against both BCSCs and non-BCSCs compared to that of the liposomes without F3 peptides.[150] Similarly, glucose transporter (GLUT) is overexpressed in many cancer cells and cancer stem cells owing to the Warburg effect.[146,186,187] Thus, GLUT is a potential target for cancer treatment. Recently, Yi et al[155] created a glucose-installed nanocarrier by conjugating unimer polyion complexes on Au nanoparticles for the targeted delivery of polo-like kinase 1 (PLK1) siRNA to BCSCs via the interaction of glucose ligands with the glucose transporter 1 (GLUT1) overexpressed on their surface. Glucose nanoparticles loaded with PLK1 siRNA have the capacity to efficiently reduce the percentage of BCSCs in cancer stem cell-rich orthotopic MDA-MB-231 tumor tissue by enhancing gene silencing, indicating that modification with glucose could significantly enhance the BCSC-targeted delivery of nanocarriers.

Surface ligand density also plays an essential role in cancer cell-specific delivery. Thus, dual-targeted NDDSs appear to be an efficient strategy for enhancing the BCSC-targeting capacity of NDDSs. For example, Qiao et al[147] developed an NDDS for the highly selective targeting of BCSCs by binding hyaluronic acid and doublecortin-like kinase 1 (DCLK1) monoclonal antibody on the surface of PEG–PLGA nanoparticles (PEG–PLGA NPs). The formed NPs were named DCLK1–HA–PEG–PLGA NPs, and they targeted BCSCs both in vitro and in vivo by the specific interaction of the DCLK1 monoclonal antibodies and HA molecules with the DCLK1 protein and CD44 receptors overexpressed on the surface of BCSCs, respectively. Similarly, nucleus-targeted drug delivery systems hold great potential to reverse cancer stem cell-mediated drug resistance. Because of this, Li et al[148] designed and synthesized a nanosystem with a core/shell structure of mesoporous silica nanoparticles loaded with the anticancer drug tirapazamine (TPZ). The surface of the silica was also modified with anti-CD133 antibody and TAT peptide. This nanosystem possessed three stages of drug delivery: 1) target BCSCs with the anti-CD133 antibody; 2) target the nucleus with the TAT peptide; and 3) release the TPZ in the nucleus to eliminate hypoxic BCSCs. It was revealed that the synthesized nanosystem significantly inhibited BCSC survival in vitro and suppressed tumor growth in a breast tumor xenograft model without obvious side effects. Mechanistically, this nanosystem was found to attenuate the hypoxia signaling pathway by suppressing the expression of HIF-1α.[148]

Advantages and Limitations of Current NDDSs Against BCSCs

The overview of the current state suggests that NDDSs are an effective treatment solution that can overcome the disadvantages of conventional therapeutic agents against BCSCs and speed up the development of potential drugs against breast cancer via anti-BCSCs. Compared to the conventional agents, NDDSs have some potential advantages against BCSCs. For example, NDDSs can overcome many of the undesirable properties of conventional agents against BCSCs.[3,130,137] Moreover, although normal stem cells and BCSCs may have similar properties, such as self-renewal,[80] NDDSs could reduce, at least to some extent, the toxicity on normal stem cells by selectively accumulating in tumor tissue with the help of the EPR effect to minimize any impact on normal stem cells.[131] In addition, the targeting capacity of NDDSs to BCSCs could be further enhanced by the modification of NDDSs with BCSCs surface marker-specific

ligands/antibodies, resulting in a further increase in the anti-BCSC ability of NDDSs while reducing toxicity on normal tissue.[131] Furthermore, NDDSs could encapsulate agents used against the large numbers of breast cancer cells, agents against the less abundant BCSCs, and/or agents targeting the breast tumor microenvironment into the same nanoparticle, enabling these drugs to target the tumor tissue as a single drug. This can solve the potential problems arising from these drugs due to the fact that they possess different biopharmaceutical parameters in vivo, which prevents them from producing the desired synergistic effect.[163] Nevertheless, the research and development of NDDSs against BCSCs is in its infancy, and many problems need to be overcome. Further research on the biological characteristics of BCSCs and the design of more efficient NDDSs is needed to overcome the limitations identified during the practical application of NDDSs. First, to eliminate BCSCs within breast cancer tissue, the targeted NDDSs need to penetrate the sites where BCSCs are located. Indeed, some subpopulations of BCSCs are located in poorly vascularized regions, which are extremely difficult for NDDSs to reach.[3] Another limitation of NDDSs is that although many solutions have been proposed to reduce the reticuloendothelial system (RES) uptake of NDDSs, the retention of NDDSs in bypassing organs and their cellular uptake by RES macrophages is still a significant problem.[163]

Conclusion

Through their involvement in the relapse, metastasis, and therapeutic resistance of breast cancer, BCSCs can make its treatment challenging. However, with a greater understanding of the biological properties of BCSCs, an increasing number of strategies, such as targeting surface markers, specific signaling pathways, metabolism, and the microenvironment of BCSCs, have been proposed to treat breast cancer by eradicating BCSCs. In this review, we summarized the current development of anti-BCSC strategies to treat breast cancer using conventional agents and NDDSs against BCSCs. Although many agents, including small-molecule inhibitors/drugs, proteins, and nucleic acids, have the potential to eliminate BCSCs, their clinical translation is limited because of their poor solubility, instability, unfavorable biodistribution, and high toxicity induced by off-target effects. This overview of the recent utilization of NDDSs to target BCSCs suggests that NDDSs have the capacity to address many shortcomings of current anti-BCSC agents and exhibit various advantages to treat breast cancer by eliminating BCSCs. However, the application of NDDSs for BCSC targeting is in its infancy, and many issues require further elucidation to develop more efficient NDDSs for targeting BCSCs with low systemic toxicities.

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Disclosure

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