Recent advances in drug delivery systems for targeting cancer stem cells

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Abstract
Cancer stem cells (CSCs) are a subpopulation of cancer cells with functions similar to those of normal stem cells. Although few in number, they are capable of self-renewal, unlimited proliferation, and multi-directional differentiation potential. In addition, CSCs have the ability to escape immune surveillance. Thus, they play an important role in the occurrence and development of tumors, and they are closely related to tumor invasion, metastasis, drug resistance, and recurrence after treatment. Therefore, specific targeting of CSCs may improve the efficiency of cancer therapy. A series of corresponding

Abbreviations: ABC, ATP binding cassette; AFN, apoferritin; ALDH, aldehyde dehydrogenase; BM-MSCs-derived Exos, bone marrow mesenchymal stem cells-derived exosomes; CAFs, cancer-associated fibroblasts; CL-siSOX2, cationic lipoplex of SOX2 small interfering RNA; CQ, chloroquine; cRGD, cyclic Arg-Gly-Asp; CSCs, cancer stem cells; DDSs, drug delivery systems; DCLK1, doublecortin-like kinase 1; Dex, dexamethasone; DLE, drug loading efficiency; DOX, doxorubicin; DQA-PEG\textsubscript{2000}-DSPE, dequlinium and carboxyl polyethylene glycol-distearylphosphatidylethanolamine; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; EpCAM, epithelial cell adhesion molecule; EPND, nanodiamond-Epirubicin drug complex; GEMP, gemcitabine monophosphate; Glu, glucose; GLUT1, glucose ligand to the glucose transporter 1; HCC, hepatocellular carcinoma; HH, Hedgehog; HIF1\textalpha, hypoxia-inducible factor 1-alpha; HNSCC, head and neck squamous cell carcinoma; IONP, iron oxide nanoparticle; iTEP, immune-tolerant, elastin-like polypeptide; LAC, lung adenocarcinoma; LNCs, lipid nanocapsules; mAbs, monoclonal antibodies; MAPK, mitogen-activated protein kinase; MB, methylene blue; MDR, multidrug resistance; MNP, micellar nanoparticle; mPEG-b-PCC-g-GEM-g-DC-g-CAT, poly(ethylene glycol)-block-poly(2-methyl-2-carboxyl-propylene carbonate-graft-dodecanol-graft-cationic ligands); MSNs, mesoporous silica nanoparticles; Nav, navitoclax; ncRNA, non-coding RNAs; NF\textsubscript{xB}, nuclear factor-kappa B; PBAEs, poly(\beta-aminoester); PDT, photodynamic therapy; PEG-b-PLA, poly(ethylene glycol)-block-poly(\omega,\omega\text{-lactide}); PEG-PCD, poly(ethylene glycol)-block-poly(2-methyl-2-carboxyl-propylene carbonate-graft-dodecanol); PEG-PLA, poly(ethylene glycol)-block-poly(\omega,\omega\text{-lactide}); PLGA, poly(ethylene glycol)-poly(\omega,\omega\text{-lactide}-co-glycolide); PTX, paclitaxel; PU-PEI, polyurethane-short branch-polyethylenimine; Salt-ABA, 4-(aminomethyl) benzaldehyde-modified Sali; SLNs, solid lipid nanoparticles; SSCs, somatic stem cells; TNBC, triple negative breast cancer; TPZ, tirapazamine; uPAR, urokinase plasminogen activator receptor.

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promising therapeutic strategies based on CSC targeting, such as the targeting of CSC niche, CSC signaling pathways, and CSC mitochondria, are currently under development. Given the rapid progression in this field and nanotechnology, drug delivery systems (DDSs) for CSC targeting are increasingly being developed. In this review, we summarize the advances in CSC-targeted DDSs. Furthermore, we highlight the latest developmental trends through the main line of CSC occurrence and development process; some considerations about the rationale, advantages, and limitations of different DDSs for CSC-targeted therapies were discussed.

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1. Introduction

Cancer is a major disease that threatens human health and life. According to WHO report on cancer 2020, 18.1 million cases and 9.6 million deaths were recorded in 2018, making cancer the second leading cause of death worldwide. The global cancer burden is increasing, and the incidence and mortality rate may nearly double by 2040\cite{1}. Although a series of treatments for tumors, including surgical intervention, chemotherapy, and radiation therapy, have been significantly refined and improved in recent years, these conventional treatments cannot adequately treat many patients, particularly those diagnosed at an advanced stage\cite{3}. Therefore, tumor recurrence and metastasis have remained a challenge\cite{4}. With research, the cancer stem cells (CSCs) theory\cite{5} could provide new insights into cancer therapy. CSCs are rare cells of tumor tissues with indefinite proliferative potential that can drive tumorigenesis. Compared with other common cancer cells, CSCs have multiple unique characteristics that are critical for cancer initiation, progression, metastasis, relapse, and drug resistance (Fig. 1\cite{6}). Firstly, CSCs possess the potential to self-renew, which makes them immortal and gives them the ability to maintain tumor masses. Secondly, CSCs have been described as a class of pluripotent cancer cells. Their behavior resembles that of normal stem cells and they can be differentiated into cancer cells with different phenotypes, leading to the progression of primary tumor and the occurrence of new tumors. Moreover, CSCs express high levels of ATP-binding cassette (ABC) transporters and are involved in the dysregulation of signaling pathway networks, which result in the acquisition of multidrug resistance (MDR) and maintenance of self-renewal properties, respectively\cite{7}.

Moreover, as postulated by the “seed-soil” theory of organ specificity for tumor metastasis\cite{8}, where “seed” refers to metastatic tumor cells and “soil” refers to organs or tissues, which provide suitable microenvironment for tumor growth, only in the appropriate “soil”, can “seeds” be colonized, proliferated, and metastasized. It is also widely assumed that stem cells exist in normal organs or tissues and dwell in a special microenvironment known as “stem-cell niche”. Various paracrine factors and direct cell contact in the stem cell niche are conducive to stem cell maintenance as well as self-renewal and differentiation pathways of stem cells in their domain. Based on the aforementioned two theories, stem cells in tumor tissues, which are referred to as CSCs, can act as “seeds” and their specific environment, which is referred to as “CSC niche”, can act as “soil”\cite{9}. In particular, the niche comprises external signals (extracellular matrix, networks of cytokines and growth factors, physicochemical factors, etc.) and various types of cells around the CSCs (fibroblastic cells, immune cells, endothelial and perivascular cells, etc.)\cite{9,10}. On the one hand, the components and unique physiological conditions within the niche form a strong external barrier to impede anti-CSC drug delivery, especially fibroblasts in the tumor microenvironment and ECM components secreted by activated fibroblasts or myofibroblasts\cite{11}. On the other hand, the niche is one of the most important tumor heterogeneity elements that drive cancer drug resistance\cite{12}. Due to the aforementioned complicated characteristics of CSCs, traditional cancer treatment regimens only kill common cancer cells with limited proliferative potential, which leads to the reduction of tumor masses but the occurrence and survival of CSCs. The remaining CSCs form new tumors under the nourishment of CSC niche after a period of proliferation and differentiation, leading to the reestablishment of tumor\cite{13}. Thus, targeting CSCs is considered a more promising approach for improved therapeutic outcomes.

Many efforts have been dedicated to CSC-targeted therapies in the past few years. Accordingly, a series of promising new therapeutic strategies to attack CSCs directly, such as CSC biomarkers-mediated targeting, targeting of CSC mitochondria, targeting of CSC genes and epigenetics, are being developed\cite{14}. Moreover, over the past 100 years, many treatments based on the “seed-soil” theory for common tumor cells, such as chemotherapy, surgical resection, and the inhibition of EGFR in tumor niche, have achieved good results\cite{15,16,17,18}. By analogy, targeting CSCs has also been carried out by targeting CSC niche outside CSCs in order to indirectly attack CSCs. The two aforementioned strategies have led to the development of many corresponding drugs\cite{19,20,21}. The effectiveness of these strategies has been confirmed to a certain extent. However, the therapeutic effects in practical applications are still far from satisfactory in view of the complex microenvironment and unique biological characteristics of CSCs. Multiple physiological barriers before reaching CSCs (except for topical administration) as well as the pharmacokinetics, bio-distribution, membrane transport properties, toxicity, and other unfavorable pharmaceutical properties of different drug molecules also limit the effectiveness of these strategies\cite{22}. The two prominent issues in CSC targeting include (1) how to convey the anti-CSC niche agents to the target CSC niche, (2) how to ensure that anti-CSC agents arrive at the target CSCs and are taken up by CSCs. Luckily, the booming drug carriers and DDSs have opened up bright prospects for tackling the aforementioned issues, paving new ways for gaining encouraging therapeutic outcomes with the aforementioned strategies and the associated drugs. The following section will systematically review recent DDS-based therapies against CSCs (Fig. 2\cite{23}) and their associated specific mechanisms\cite{24,25,26,27,28,29,30,31} (Figs. 3–7). Furthermore, it will discuss the
current prospects and the challenges associated with these targeted therapies.

2. DDSs for targeting CSC niche

Increasing evidence shows that the CSC niche dominates the self-renewal and differentiation of CSCs. Moreover, they can induce the differentiation of tumor cells with CSC characteristics by receiving signals from CSC microenvironment or through stem gene activation. As such, the non-CSCs become new CSCs, resulting in the initiation and progression of new CSCs. The newly formed CSCs can differentiate into cancer cells, forming a vicious circle. Moreover, organs with CSC niche-like characteristics are more likely to accept disseminated tumor cells due to a pre-built conducive microenvironment, also known as premetastatic niche, which promotes tumor cell dissemination and invasion. In this complex niche, whether in a primary or secondary niche, CSCs exhibit significant phenotypic and functional heterogeneity, and their progenies exhibit different plasticity. This may be another Darwinian selection during tumor progression. Fortunately, unlike CSCs which have high plasticity, the CSC niche and its various components and properties are highly conserved in the process of biological evolution, and tumors of different origins, genotypes, and histology share common microenvironmental elements, and hence, targeting the CSC niche can be regarded as a rational choice. Targeting the CSC niche or its various components can be achieved by nano-DDSs. These nano-DDSs could deliver anti-CSC niche agents to the target CSC niche through the enhanced permeability and retention effect (EPR)-effect. In addition, some components within the niche can serve as a stimulus and mediate a smart targeting delivery. Over the past several years, there has been a speeding progress in the development of omnidirectional CSC niche-targeted DDSs, some of which are targeted at preventing the formation of CSC niche and inhibiting the development and metastasis of CSC niche that has already been formed.

Based on the hypoxic microenvironment of CSCs, a polymer–surfactant nanoparticle (NP) system was developed with sodium alginate and docusate sodium (Aerosol OT, AOT) to encapsulate methylene blue (MB), a widely used photosensitizer. Upon light stimulation, the resultant MB-NPs significantly decreased the formation of primary and secondary mammospheres, the number of colonies in soft agar, and aldehyde dehydrogenase (ALDH)-positive cells, which suggest that photodynamic therapy (PDT) with MB-NPs is an efficient approach against CSCs. Likewise, more NP-mediated oxygen carrying and oxygen generation to relieve cancer niche hypoxia were summarized by Wang et al., including PFC- and Hb-based oxygen-carrying NPs, self-decomposed and photocatalyst-based oxygen-generating NPs.

Epithelial-mesenchymal transition (EMT) is another crucial and early step in the induction of the formation of a CSC niche and CSCs. Once cancer cells undergo EMT, they show similar characteristics to CSCs, such as increased drug efflux pumps and enhanced anti-apoptotic effects. After EMT, these cells may temporarily enter dormancy and no longer divide. Therefore, we can conclude that EMT probably triggers CSC generation, and targeting EMT is likely to have great potential in preventing CSC formation by interfering with its development in the CSC niche. Chiu et al. used polyurethane-short branch-polyethylenimine (PU-PEI) as a carrier to deliver miR145 into lung adenocarcinoma CSCs (LAC-CSCs). The authors observed successful delivery of miR145 as well as reduced CSC-like properties and tumor growth and metastasis, which was most likely a consequence of inhibited EMT. Ahmad et al. developed a dexamethasone (Dex)-associated liposome (DX) for the delivery of anticancer drug ESC8 and NRP-1 shRNA-encoded plasmid to breast cancer stem-cell-like cells ANV-1 (DXE-NRP-1). Treatment with DXE-NRP-1 led to significant down-regulation of EMT markers, including Id-1 and α-SMA, and SNAI-1, a suppressor of E-cadherin (epithelial marker), which promoted the sensitization and killing of highly aggressive and drug-resistant CSCs.

Signaling pathways also play an unparalleled role in the CSC niche. CSCs and normal somatic stem cells (SSCs) share common signaling pathways in order to maintain their stem cell-like characteristics. The differences between CSCs and SSCs are most likely due to one or more abnormalities in the various signaling pathways of CSCs, which is specifically reflected as the up- or down-regulation of biomolecules or their receptors in the signaling pathways. The top three most important signaling pathways related to self-renewal are the WNT/β-catenin, NOTCH, and Hedgehog (HH) pathways. Furthermore, increasing evidence indicates that these pathways can interact with other cellular signaling pathways such as the NF-κB, MAPK, PI3K, and EGF pathways.
pathways play a regulatory network role in the CSC niche. Accordingly, regulating the signaling pathway in different ways and at different levels may achieve a multiplier effect for the complete elimination of CSCs. Karandish et al. encapsulated napabucasin, a cancer stemness inhibitor, with synthesized iRGD-targeted polymersomes. They reported decelerated cell viability of both prostate and pancreatic CSCs as well as significantly decreased expression of cancer stemness markers, such as NOTCH-1 and NANOG, which indicates the inhibitory effect of the iRGD-targeted polymersomes against cancer stemness. Liu et al. developed U0126-loaded NPs (NPU0126) for both bulk hepatocellular carcinoma (HCC) and HCC CSC therapy with U0126, a dual functional mitogen-activated protein kinase (MAPK) inhibitor, as the drug and poly (ethylene glycol)-b-poly (D,L-lactide) (PEG-PLA) as the carrier. With NPU0126 treatment, higher therapeutic efficacy and lower systemic toxicity were achieved, which was not only reflected in the substantial reduction of sphere formation in all tested cell lines, but also in the CSC populations (CD133-positive). These results indicate that NPU0126 significantly inhibited CSC self-renewal and interfered with CSC stemness. In a bid to further improve CSC targeting, Miller-Kleinhenz et al. developed a dual targeting iWNT-ATF24-ultra-small magnetic iron oxide NP (IONP), which was embellished with both iWnt and ATF24 targeting peptide, urokinase plasminogen activator receptor (uPAR). Cell assay results showed that iWnt-ATF24-IONP-DOX simultaneously and effectively downregulated the WNT/β-catenin pathway, uPAR expression, and CSC-associated biomarkers, resulting in inhibited cell invasion and proliferation of CD44+/CD24−/low cancer stem-like cell population. Similar results were obtained in an orthotopic chemoresistant breast cancer PDX model in vivo.

In addition, there were other DDSs or carriers designed for targeting ECM and cancer-associated fibroblasts (CAFs) within the niche. Goodman et al. used collagenase-coated polystyrene NPs to lead collagenase into the CSC niche, thereby degrading the ECM. Chen et al. reported the development of a novel tumor stroma-targeted nanoparticle system to achieve targeted delivery of Navitoclax (Nav), an anti-CAF drug, to CAFs. Despite the effectiveness of the CSC niche-based DDSs, targeting only the CSC niche is inadequate to eliminate CSCs. Exploring the advantages of CSC niche-targeting therapies and combining with other strategies to achieve the complete elimination of CSCs is worth exploring. A typical example is the combination of paclitaxel (PTX) and gemcitabine monophosphate (GEMP)-loaded bone marrow mesenchymal stem cell-derived exosomes (BM-MSC-derived exosomes).

3. DDSs for direct targeting of CSCs at the cellular level

3.1. Transforming traditional anticancer drugs into CSC killers through CSC biomarkers-mediated delivery systems or well-designed DDSs

At present, complete identification and isolation of CSCs are still difficult for researchers. However, it is possible to differentiate CSCs from other common cancer cells and normal stem cells on the strength of their specific surface biomarkers. There have been quite a lot of biomarkers described, such as CD44, CD133, and epithelial cell adhesion molecule (EpCAM). The strong binding between the existing biomarkers and their specific antibodies provides enhanced targeting based on the EPR effect, which is accompanied by a high cellular uptake and increased drug concentration in CSCs. In this respect, targeting CSCs based on their specific biomarkers is unquestionably a rational choice, and correspondingly, a great amount of biomarkers-
mediated delivery carriers and systems have been developed and explored. Table 2 presents an overview of recent representative drug delivery carriers and systems. CD44 is one of the most famous surface markers related to CSCs, and HA is the main component of the extracellular matrix, especially as it is abundantly expressed in a wide variety of CSCs and has a high affinity for CD44 receptors and better biocompatibility than anti-CD44 antibody. In respect of this, Shen et al. developed a solid lipid NP delivery system coated with hyaluronic acid (HA-SLNs) to encapsulate PTX through the film-ultrasonic method. The prepared cationic HA-SLNs/PTX achieved a mean diameter of 160.6 ± 1.997 nm, a poly disparity index of 0.218 ± 0.012, and a zeta potential of 46.3 ± 1.07 mV. The uptake experiments showed that HA-mediated mechanism led to higher cellular uptake of HA-SLNs/PTX than that of SLNs/PTX lacking HA and heparin-SLNs/PTX coated with similar mucopolysaccharide heparin (Fig. 3A). Based on the results, the lowest half maximal inhibitory concentration (IC₅₀) (11.13 ± 1.62 μg/mL) was achieved by HA-SLNs/PTX, whereas the IC₅₀ values of SLNs/PTX and free PTX were up to 18.11 ± 3.79 and 31.39 ± 4.81 μg/mL, respectively. Consistent results were found in A549 cells. Furthermore, treatment with HA-SLNs/PTX reduced the proportion of side population cells in B16F10-CD44⁺ cells from 56.2% to 6.7%, whereas PTX-loaded SLNs reduced the proportion to 14.5%. Moreover, after treatment with HA-SLNs/PTX, the expression of Oct-4 in B16F10-CD44⁺ cells was significantly reduced. Taken together, these findings suggest that HA-SLNs/PTX represents a preferential strategy for anti-CSC therapy. Yi et al. developed a glucose-installed sub-50-nm gold NPs (Glu-AuNPs) through a two-step self-assembly. The constructed Glu-AuNPs successfully condensed siPLK1, an important gene responsible for cell cycle, to protect it from degradation. It achieves CSC targeting by reaching, recognizing, and combining with its specific receptor glucose transporter 1 (GLUT1) overexpressed on the CSC surface. Because of the specific binding between the Glu ligands and GLUT1, the siPLK1-loaded Glu-Au NPs presented higher cellular uptake, accompanied by higher gene silencing efficiency and better anticancer activity both in the GLUT1-overexpressing MDA-MB-231 cell spheroids and MDA-MB-231 orthotropic tumor (Fig. 3B). Similarly, Ning et al. fabricated PEG-PCL-based NPs conjugated with anti-CD133 antibody to effectively deliver SN-38, a topoisomerase inhibitor, to target CD133-positive cells through receptor-mediated endocytosis, and they observed the same cytotoxic effect on CSCs as the siPLK1-loaded Glu-Au NPs (Fig. 3C). Li et al. proposed the use of a mesoporous silica NP-based nucleus-targeted nanodelivery system to deliver tirapazamine (TPZ) (CD133/TAT/TPZ-Fe₃O₄@mSiO₂ NPs), an anticancer drug, to hypoxic CSCs. First, as TPZ plays its role mainly in the nucleus, the constructed CD133/TAT/TPZ-Fe₃O₄@mSiO₂ NPs positively targeted CSCs through receptor-mediated endocytosis. Second, nucleus-targeting was achieved by TAT peptide, which escorted TPZ directly to the nucleus to exert its effects. Third, the innermost layer of the Fe₃O₄ NPs, which in turn attenuated the hypoxia-inducible factor 1-alpha (HIF1α) by CD133/TAT/TPZ-Fe₃O₄@mSiO₂ NPs, which in turn attenuated the hypoxia-signaling pathway, led to the elimination of CSCs. Miyano et al. incorporated cisplatin into a cyclic Arg-Gly-Asp (cRGD) peptide-installed micellar nanomedicine (cRGD-CDDP/m). Owing to the CSC subpopulation in head and neck squamous cell carcinoma (HNSCC) cells overexpressing αvβ₅ integrins and cRGD peptide, cRGD-CDDP/m could successfully exert its inhibitory activity against recalcitrant HNSCC CSCs. SAS-L1-Luc cells with superior levels of αvβ₅ integrins and CD44v9 were selected to evaluate the micelles. In vitro cytotoxic effects showed that cRGD-CDDP/m significantly decreased the proportion of CD44v9-positive SAS-L1-Luc cells at low or high doses. Further experiments demonstrated that the effect was due to EPR effect-mediated penetration, vascular targeting, and interference with tumor metastasis in the lymphatic system of cRGD-CDDP/m.
Figure 4  Schematic illustration of mertansine-apoferritin (M-AFN) preferentially taken up by cancer stem cells (CSCs)-enriched tumorspheres. Reprinted with the permission from Ref. 27. Copyright © 2018 Elsevier B.V.

Figure 5  Schematic illustration of mitochondria-targeted delivery. Reprinted with the permission from Ref. 28. Copyright © 2010 American Chemical Society.

Figure 6  Schematic illustration of R646 nano-miRs targeting multiplexed cancer stem cells-regulating miRNAs. Reprinted with the permission from Ref. 29. Copyright © 2018 American Chemical Society.
Therefore, there is no doubt that in the fight against HNSCC, cRGD-CDDP/m shows good prospects. However, by targeting one cell surface marker, the aforementioned DDSs are not sufficient to precisely target and kill all CSCs because of the overlap of the biomarkers. Qiao et al. prepared poly (ethylene glycol)-poly (D,L-lactide-co-glycolide) (PLGA) NPs with HA and doublecortin-like kinase 1 (DCLK1) monoclonal antibody to target CD44 and DCLK1 surface marker, respectively. Both in vitro and in vivo results indicated that DCLK-HA-PEG-PLGA NPs could target CSCs with high efficacy. Furthermore, many researchers have suggested that well-designed DDSs are also a powerful aid in transforming conventional chemotherapeutic agents into CSC killers (Table 3). Tan et al. used apoferritin, a material that could be preferentially recognized and internalized by CSCs, to load mertansine (M-AFN), a highly cytotoxic agent for tumors, to effectively target CSCs. The results validated the fact that M-AFN was taken up, and it subsequently exerted an inhibitory effect on CSC-enriched tumorsphere cells. The above satisfactory therapeutic effect could be attributed to its ability to prioritize CSCs and its pH-sensitive drug release performance, as depicted in Fig. 4. Sun et al. reported a gold NP-based DDS (DOX-Hyd@AuNPs) to mediate potent delivery of doxorubicin (DOX), which was achieved by connecting a gold NP surface poly (ethylene glycol) spacer with DOX through acid-labile linkages. Compared with free DOX, DOX-Hyd@AuNPs induced more effective delivery of DOX to breast CSCs and subsequent greater reduction of the regenerated mammospheres, indicating that the stemness of CSCs was significantly decreased and tumor growth was effectively inhibited. Although epirubicin and nanodiamonds can reversibly adsorb and desorb, Wang et al. used a nanodiamond-drug delivery platform, nanodiamond-epirubicin drug complex (EPND), to deliver epirubicin. In vitro experiments demonstrated that EPND could prolong the retention time of epirubicin in tumor cells and

Table 1 Drug delivery systems and carriers for targeting CSC signaling pathways.

| Signaling pathway | Drug | Carrier and feature | Cell line | Ref. |
|-------------------|------|---------------------|-----------|------|
| Stemness          | Napabucasin | iRGD peptide-decorated, reduction-sensitive polymersomes | Human prostate stem cells, human pancreatic cancer stem cells | 48 |
| MAPK              | U0126 | PEG-PLA NPs | HepG2, Hep3B, SMMC-7721 | 49 |
| WNT               | DOX, iWnt and ATF2 | Dual receptor targeted iWnt-ATF2-IoNP | MDA-MB-231 | 50 |
| STAT3             | Niclosamide | Active targeting A15-SLNs | OSCC cells | 51 |
| Proteasome        | CGX1321 | PEG-b-PLA NPs | Hek293, LoVo | 52 |
| PI3K/AKT          | BTZ | PEG-coated GNP and cold plasma | MDA-MB-468, HCC1937 | 53 |
| P53/P21 dependent manner | QAuNP | Cationic lipid-assisted polymeric NPs | A549, T98G | 54 |
| TGF-β             | LY364947 and siPik1 | Triblock structured PM based on the combination of PEI with Pfurionic amphiphilic copolymers | H-357-PEMT | 55 |
| P3K               | CDF | Liposome | MDA-MB-231 | 56 |
| AKT independent pathway | DAPT | C/D targeting PEI-GA NPs | MCF-7, MDA-MB-231 | 57 |
| NOTCH             | DAPT | MSN-PEI-GA NPs | MDA-MB-231 | 59 |
| FER               | FER siRNA | Liposome | MDA-MB-231 | 60 |
| β-Catenin         | LincRNA-P21 | Liposome | HCT116 | 61 |
effectively target chemoresistant CSCs, leading to significant reduction in the percentage of both non-side and chemoresistant side populations. The in vivo analysis results are consistent with the in vitro experiments. Zhao et al.\textsuperscript{96} proposed that SP1049CM (now code-named SKC1049), a DOX-containing polymeric micelle formulation of a mixture of Pluronic L61 and F127, could eradicate CSCs in triple negative breast cancer (TNBC). Du et al.\textsuperscript{97} designed a tailor-made dual pH-responsive polymer-DOX conjugate (PPC-Hyd-DOX-DA), which drastically inhibited the progression of drug-resistant SK-3rd CSCs. Taken together, biomarkers-mediated delivery systems or well-designed DDSs have achieved encouraging results in CSC-targeted therapies. However, the development of improved methods to identify optimal biomarkers and the best combination of biomarkers and different DDSs need to be explored.

3.2. DDSs for autophagy-mediated targeting

In order to cope with various ambient stresses such as nestia, radiation, hypoxia, and chemotherapy drugs, the body itself has evolved a conservative physiological process known as autophagy. Autophagy is a process whereby substances such as organelles, proteins, and RNAs need to be degraded in the cytoplasm and cell form autophagosomes and are degraded by autophagy lysosome in order to maintain balance and reduce metabolic stress\textsuperscript{101}. Owing to it being a physiological process of death that relies on its own lysosomes and forms a characteristic structure named autophagosome, it is referred to as “type II programmed death.” Similar to apoptosis, “type I programmed death”, it is instrumental to cell growth, differentiation, and the maintenance of homeostasis\textsuperscript{101}, and because of this, many researchers have been attracted to research in the field of cancer autophagy. Interestingly, some studies have indicated that autophagy is indispensable in all stages of CSC physiology, including generation, differentiation, plasticity, migration/invasion, and pharmacological, viral, and immune resistance. Therefore, targeting autophagy could open a new way to deal with CSCs\textsuperscript{102}. Recently, several scientists reported that chloroquine (CQ) could inhibit autophagy, and nano DDSs together with CQ have enormous potential for improved cancer treatment\textsuperscript{103}. Sun et al.\textsuperscript{106} encapsulated DOX and DTXL with CQ in poly(ethylene glycol)-block-poly(D,L-lactide) (PEG-b-PLLA) NPs (denoted as NPCQ/NPDOX and NPCQ/NPDTXL, respectively) through single emulsification. The diameter was around 110 nm and the drug loading efficiency (DLE) varied between 50.2\% and 65.8\%. Moreover, within the concentration ranges for cell culture and animal research, both NPCQ/NPDOX and NPCQ/NPDTXL could be properly and uniformly dispersed in aqueous solution. Sorted ALDHhi MDA-MB-231 cell tests indicated that the administration of NPCQ/NPDOX and NPCQ/NPDTXL particularly optimized the level of ALDHhi MDA-MB-231 cells and succeeded in hindering mammosphere formation and tumor enlargement. Encouragingly, tumor growth was remarkably impeded, and NPCQ/NPDOX and NPCQ/NPDTXL produced a strong inhibitory response against CSC subpopulation in an MDA-MB-231 orthotropic tumor murine model.

3.3. DDSs for immune-mediated targeting

Immunotherapy has recently become the focus of global attention and has become a “new hope” for cancer treatment, especially after the announcement of The Nobel Prize in Physiology or Medicine 2018, which involved the development of cancer therapy through the suppression of negative immune regulation\textsuperscript{107,108}. In light of less sensitivity of CSCs to conventional methods and the

| Surface biomarker | Therapeutic agent | Carrier and feature | Cell line | Ref. |
|------------------|------------------|-------------------|-----------|-----|
| CD44v6           | GNS-PEG-CD44v6   | with NIR          | MKN-45    | 84  |
| HER2             | Sali             | NP-HER2           | MDA-MB-361, BT-474 | 85  |
| CD20             | SA               | CD20-SA-NPs       | WM266-4, A375 | 86  |
| Sigma-2 receptor | DOX              | SV119-PEG-AuNCs   | MDA-MB-435 | 87  |
| CD44 and DCLK1   | DOX              | DCLK1-PEG-PLGA NPs | 4T1      | 88  |
| CD44 and integrin αβ3 | mTRAIL plasmid   | RRPHC ternary complexes | B16F10 | 89  |
| EGFR and CD133   | Sali             | CESP              | Saos-2, MG-63 | 90  |

Table 2 Targeting CSCs by biomarkers-mediated carriers and drug delivery systems.
Exciting effect of the immune system, including innate and adaptive immunity, a wide variety of immunotherapy-based approaches have been developed to target CSCs. EI-Ashmawy et al. prepared a CSC-DC-based vaccine against CSCs, which was developed by incorporating antigens harvested from drug-resistant cancer cells with a CSC-like phenotype into DCs. The results illustrated that the CSC-DC-based vaccine markedly increased the serum IFN-γ level and up-regulated p53 expression, which indicate effective anti-tumor immune responses. Finally, co-treatment with this vaccine and low doses of cisplatin achieved a high inhibitory effect on the proliferation of CSCs. The same result was reported by Dashi and his partners. In addition to CSC-based vaccines, numerous monoclonal antibodies targeted directly at CSC surface biomarkers are involved in immunotherapy. Bourreau-Guilmain et al. used surface-modified monoclonal antibody lipid nanocapsules (LNCs) to simultaneously target and deliver AC133 antibody to CSCs. Due to the fact that AC133 is the most prominent marker related to CSC phenotypes, an excellent binding capacity to CSCs was observed.

4. DDSs for targeting CSC organelles

The mitochondrion is one of the most important organelles, which participates in the whole process of cell fate determination and development, from cell self-movement to cell signaling and cell death. As mentioned earlier, CSCs show abnormalities in metabolism, proliferation, and apoptosis. Therefore, it is not surprising that CSCs appear to experience pathological reprogramming, particularly the remodeling of mitochondrial functions. The role of mitochondria as a central hub has led to the development of mitochondria-targeted anti-CSC therapeutic strategies to combat cancer. Studies on the deep understanding of mitochondrial biology in CSCs have been systematically elaborated, and so do the corresponding drugs that can effectively target mitochondrial functions. The successful achievement of mitochondria-targeted delivery has become the focus of many studies. A series of explorations have been made, and the answers to the associated questions are shown schematically in Fig. 5.

Wang et al. investigated if serum protein-coated Au NRs had a favorable effect on carcinoma cells. As expected, the Au NRs effectively targeted mitochondria because of enhanced liposome membrane permeation ability, reduced cancer cell efflux, and outstanding lysosomal to mitochondrial transfer ability. The parallel molecular mechanism and in vitro cytotoxicity were determined, and the results showed reduced mitochondrial membrane potential, enhanced oxidative stress, and decreased viability of cancer cells. These findings provide appropriate implications and guidance for the design of mitochondrion-targeted anti-tumor therapy. Zhang et al. constructed mitochondria-targeted quinacrine liposomes featuring dequalinium on the liposome surface as a targeted modification. Due to the mitochondrial membrane potential, the dequalinium-modified quinacrine liposomes were enriched in the mitochondria of living cells with dequalinium’s positive charge and delocalized charge center. The results showed that dequalinium-modified quinacrine liposomes exerted significantly enhanced inhibitory effect against MCF-7 CSCs. Furthermore, the significant activation of pro-apoptotic BAX protein, reduction of mitochondrial membrane potential, release of cytochrome C by translocation, and initiation of the cascade reaction of caspases nine and three induced by the aggregation of drugs into the mitochondria were clearly observed. Ma et al. developed a targeting berberine liposome, which was generated using a mitochondria-tropic functional material conjugated by dequalinium and carboxyl polyethylene glycol-distearylphosphatidylethanolamine (DQA-PEG2000-DSPE). The targeting berberine liposomes were properly transported across CSCs, and they selectively assembled in the mitochondria, thus resulting in an increased release of cytochrome C and further apoptosis of breast CSCs.

5. DDSs for targeting CSC genes and epigenetics

Initially, it was believed that all cancers are identical in pathogenesis. Currently, it is widely believed that tumors are caused by the accumulation of a series of proto-oncogenes and tumor suppressor gene mutations. Continuous acquisition of inheritable genetic variation and natural selection are two continuous processes that collectively create cancer phenotypic diversity. In fact, with the development of the concept of epigenetics over the past several years, increasing evidence indicates that epigenetics also play profound and ubiquitous roles in the pathogenesis of cancer. Epigenetics, specifying “stable heritable phenotypes due to changes in chromosomes but no changes in DNA sequences”, include DNA methylation, histone modifications, and non-coding RNAs (ncRNA). ncRNAs have critical roles in CSC biology, and hence strategies that involve the modulation of the expression of corresponding genes and ncRNAs in CSCs for CSC-targeted therapy bring enormous opportunities. However, in vivo delivery of DNA and miRNAs faces various obstacles due to their poor biological stability, short half-life, poor oral bioavailability, and inappropriate intracellular release properties and other unfavorable factors. Therefore, several suitable delivery carriers and systems have been developed. Table summarizes recent DDSs for targeting CSC genes and epigenetics.

Ke et al. delivered nuclear factor-kappa B (NF-κB) shRNA, an important gene involved in the maintenance of CSC properties, especially in various breast cancer cell lines, by using carbamate-mannose modified PEI. The results showed that the prepared non-viral gene vector decreased the ALDH+ cell population by as much as 7.9%. In addition, inhibited colony and mammosphere-forming ability as well as cell migration and invasion were
observed. Andey et al.\textsuperscript{129} prepared a cationic lipoplex encapsulating SOX2 small interfering RNA (CL-siSOX2) to specifically target SOX2-enriched, H1650 CSCs-derived lung tumors. CL-siSOX2 significantly inhibited the expression of stemness markers in xenograft tumors, such as SOX2, NANOG, c-MYC, and KLF4, and effectively shrank tumor volume in mice, and these effects were attributed to the crucial role of SOX2 in the regulation of signaling pathways associated with CSCs.

As for ncRNA, particularly miRNA, there are two possible models of its relationship with tumorigenesis in tumor cells. One is that if the expression of miRNA is up-regulated, there is a good chance that the expression of tumor suppressor genes is down-regulated, which may facilitate tumorigenesis. The other is that if the expression of miRNA is down-regulated, there is a good chance that the expression of corresponding oncogenes is up-regulated, which may also cause the development of tumors\textsuperscript{142}. Therefore, the development of therapies against CSC-based miRNAs mainly involves two aspects: restoring the expression of tumor suppressor miRNAs through miRNA analogs and inhibiting the expression of oncogenic miRNAs by miRNA antagonists or inhibitors\textsuperscript{143}. Lopez-Bertoni et al.\textsuperscript{29} combined bioreducible poly (β-aminoester) NPs (R646) with two newly discovered CSC-inhibiting miRNAs, miR-148a and miR-296-5p, to self-assemble polymeric NPs containing miRNAs. As shown in Fig. 6, the bioreducible R646 nano-miRs carrying miR-148a + miR-296-5p (Comb) effectively and simultaneously delivered miR-148a and miR-296-5p into the cells. Subsequent \textit{in vivo} experiments showed that sphere-forming capacity and tumor burden were significantly reduced and tumor cell death was observed. Therefore, it is imperative to reverse the situation by delivering small molecules with similar functions to corresponding miRNAs instead of miRNAs. Wen et al.\textsuperscript{122} packed PTX and 2'-hydroxy-2,4,4',5,6'-pentamethoxychalcone (rubone), a small-molecule modulator that up-regulates miR-34a, into polymeric micelles that were synthesized using poly(ethylene glycol)-block-poly(2-methyl-2-carboxyl-propylene carbonate-graft-dodecanol) (PEG-PCD), and rubone synergistically enhanced the therapeutic efficacy of PTX-resistant prostate cancer.

Regardless of the targeted part of CSCs, some studies pay more attention to targeting CSCs at the molecular level. However, before the DDSs arrive at the corresponding organelles or the cytoplasm, they need to be taken up by CSCs. Therefore, DDSs that are more sophisticated are needed to achieve double targeting at the cellular and molecular level.

### Table 4 Drug delivery systems for targeting CSC corresponding genes and miRNAs.

| miRNA or gene | Drug | Carrier | Cell line | Ref. |
|---------------|------|--------|----------|-----|
| miR-148a and miR-296-5p | miR-148a and miR-296-5p | Bioreducible R646 NPs | GBM1A | 29 |
| NF-κB | NF-κB shRNA | CMP | 4T1 | 128 |
| SOX2 | siSOX2 | CL | H1650 | 129 |
| miR-205 | miR-205 | mPEG-b-PCC-g-GEM-g-DC-g-CAT | MIA PaCa-2R, CAPAN-1R | 130 |
| miR-34a | miR-34a | PLA NVs | MKN-74 | 131 |
| miR-34a | Rubone | PEG-PCD micelles | DU145, PC3 | 132 |
| Gli | Antho | PLGA-NPs | AsPC-1, Mia-Paca-2, PANC-1 | 133 |
| Anx2 | shAnx2 | CLG | H1650 | 134 |
| Bmi1 | Bmi1siR | NPC | HepG2 | 135 |
| miR145 | miR145 | PU-PEI | GEM-C133* cells | 136 |
| Let-7 miRNA and CDK4 | let-7 miRNA and CDK4-specific siRNA | PEGylated liposome conjugated with heparitin | SK-BR-3 | 137 |
| Let-7a | Let-7a | PU-PEI-NLS | AA-isoalted SP* cells | 138 |
| miR-200c | miR-200c | Cationic SLN | MCF-7 | 139 |
| miR-let-7b | miR-let-7b | UTMD | A2780 | 140 |
| P70S6K | P70S6K siRNA | G0 dendriplexes | SKOV-3, HEY-A8 | 141 |

### 6. DDSs for combination therapy

It has been demonstrated that combinational treatment is superior to single treatment modalities for cancer therapy. Therefore, it is wise to combine conventional anticancer agents and anti-CSC agents for improved therapeutic response\textsuperscript{144,145}. Recently, many researchers have reported successful development of co-delivery systems for combination therapy (Table \textsuperscript{50,31,73,146–175; Fig. 7,30,31}). Zhao et al.\textsuperscript{146} developed an immune-tolerant, elastin-like polypeptide (iTEP)-conjugated NPs to co-deliver 4-(aminomethyl) benzaldehyde-modified Sali (Sali-ABA) and PTX, which were denoted as iTEP-Sali-ABA NP and PTX NP, respectively. They reported that a combination therapy with the two NPs suppressed primary tumor growth and metastasis. In addition, the combined use of the two NPs achieved longer survival than iTEP-Sali-ABA NP alone. Shen et al.\textsuperscript{147} adopted a micellar NP (MNP) to co-deliver a platinum (IV) prodruk and NOTCH1-targeting siRNA (Pt\textsuperscript{IV}/MNP/siNotch1) to treat CSCs-harboring HCC. The results showed that Pt\textsuperscript{IV}/MNP/siNotch1 efficiently deliver Pt (IV) and siNotch1 into both bulk non-CSC and CSC populations of SMMC7721, an HCC cell line, and SMMC7721 xenograft model. In addition, Pt\textsuperscript{IV}/MNP/siNotch1 significantly reduced the stemness of SMMC7721 cells,
down-regulated NOTCH1, and enhanced the cytotoxicity of
cisplatin, thus eliminating both the bulk cancer cells and the rare
CSCs.

7. Conclusions and future perspectives

The recent in-depth refinement and improvement of treatments
modalities for tumors have led to significant control of tumors as
well as extended patient survival. However, these treatments are
temporary control strategies for both metastases and primary tu-
mors, and there is a high chance of recurrence and metastasis in
patients after treatment, which can be ultimately attributed to
incomplete elimination of CSCs. Therefore, the development of
treatment regimens targeted at CSCs as well as DDSs and carriers
has attracted a lot of attention. In this review, we systematically
discussed various DDSs for CSC-targeted therapies. The afore-
mentioned DDSs and carriers effectively ameliorated the short-
comings of the physical and chemical properties of the associated
drugs, enhanced drug targeting and pharmacological activities,
and reduced the incidence of side effects, leading to the reduction
or elimination of CSCs. However, the clinical implementation of
these strategies still has a long way to go. Moving forward, further
studies should focus on the following aspects: (1) more new co-
delivery drug systems should be designed and developed to
achieve the synergistic therapeutic effect of combined drugs. The high
resistance, high metastaticity, and complex niche of CSCs make
the combination of two targets or multiple targets necessary to
achieve synergy. However, the most common co-delivery drug
systems focus on simple co-loading and neglect the pharmacoki-
netic characteristics, inhibitory effects, release performances, and
the drug—drug interactions of the different drugs within the car-
riers, which make it difficult to achieve synergistic effects. (2)
According to the different target positions of each drug, such as
CSC niche and CSC organelles, the corresponding carrier needs to
be refined to release drugs at the corresponding position to achieve
maximum efficacy and produce minimal side effects. (3) The
superior carriers should target both cancer cells and CSCs
simultaneously. According to the “seed-soil” theory, CSC prolif-
erate, move to another suitable niche with CSC niche-like fea-
tures, and subsequently reestablish their own CSC niche. The
existing CSC niche is conducive to the survival of CSCs, and the

| Co-delivered payload | Drug against cancer cells | Drug against CSCs | Carrier and feature | Cell line | Ref. |
|----------------------|---------------------------|-------------------|-------------------|----------|-----|
| DOX                  | miR-21i                   | NIR responsive HGNPs | MDA-MB-231, MCF-7 | 30       |
| PTX                  | CUR                       | pH multistage responsive PPBV | MCF7 | 31 |
| DTX                  | 8-HQ                      | HA-MSS            | MCF7              | 73       |
| PTX                  | Sali-ABA                  | tTEP NPs         | 4T1               | 146      |
| Platinum (IV)        | siNotch1                  | MNP               | Hep3B, SMMC7721   | 147      |
| DOX and SN38         |                           |                   |                   |          |
| DOX                  | ATRG                      | PEG-b-PLA NPs     | MDA-MB-231        | 149      |
| DOX                  | SAL                       | cMLV              | 4T1, MDA-MB-231   | 150      |
| PTX                  | CYP                       | mPEG-b-PCC-g-DC self-assembled into micelles | DU145, PC3, DU145-TXR, PC3-TXR | 151      |
| PTX                  | SAL                       | pH-responsive SWCNT-PEG conjugated with CD44 mAbs | MDA-MB-231 | 152 |
| Cisplatin            | HNF4α-encoding plasmid    | PMSNs             | Huh7 cells        | 153      |
| GEM                  | miR-345                   | Temperature and pH-responsive DDND | Capan-1, CD18/HPAF-II | 154       |
| PTX                  | miR-34a                   | SLNs              | B16F10            | 155       |
| Epi                  | STS                       | pH-sensitive polymeric micelles | 4T1 | 156 |
| DTX                  | CYP                       | HPMA copolymer    | PC-3, RC92a/TERT  | 157      |
| Paclitaxel           | Arteether                 | Liposome modified with MAN-TPGS1000 and DQA-PEG3000-DSPE | C6 | 158 |
| Epi                  | STS                       | pH-triggered polymeric micellar nanomedicines | MSTO-211H cells | 159 |
| PTX                  | SAL                       | Oct-modified PEG-b-PCL micelles | MCF-7 | 160 |
| Ptx                  | SAL                       | SF-NPs           | H22               | 161       |
| CDDP                 | DMC                       | CHC/anti-CD133 NPs | A549-ON           | 162       |
| EPI                  | MET                       | PEGylated liposomes | S180              | 163       |
| DOX                  | THZ                       | PEG-PUC/PEG-PAC MM | BT-474, MCF-7     | 164       |
| PTX                  | SLM                       | HA-PLGA NPs      | MCF-7, MDA-MB-231 | 165      |
| DTX                  | RUB                       | pH and GSH dual sensitive polymeric micelles | DU145-TXR, PC3-TXR | 166 |
| DXR                  | C6-Cer                    | F3 peptide-targeted liposome | MCF-7, MDA-MB-231 | 167 |
| DOX                  | NVP                       | HMSN-COOH        | CD117/ CD44/ A2780 | 168       |
| UA                   | Bmi1 siRNA                | FA-liposome       | KB cells          | 169       |
| Docetaxel            | Salinomycin               | PLGA-PEG NPs     | MKN-45, NCI-N87   | 170       |
| CBX                  | SIL                       | HA-coated liposomes | PC-3, DU-145    | 171       |
| PTX                  | TS prodrug conjugate      | HTS NPs          | MCF-7             | 172       |
| DOX                  | CYC                       | HA-SS-PLGA NPs   | MCF-7, MDA-MB-231 | 173      |
| PTX + HY             | THZ                       | PM@THL           | MCF-7             | 174       |
| CPT and PTX          | cMLV                      |                   |                   |          |
appropiate niche in the distance promotes the transfer of CSCs. The two processes play a complementary role and promote each other. Moreover, CSCs can differentiate into cancer cells, and cancer cells can be induced to transform into CSCs, forming a dynamic reciprocal equilibrium process. Hence, to achieve the thorough treatment of cancers, cancer cells and CSCs should be targeted and killed, and the CSC niche should be simultaneously destroyed. The number of CSCs in cancer tissue is small, and hence, a large number of existing normal cancer cells should first be removed, followed by the targeting of the exposed CSCs for elimination. (4) In the context of personalized medical practices and the complex causes of CSCs, it is crucial to integrate the understanding of the underlying pathogenesis of cancer in individual patient so that the appropriate corresponding drug or strategy and drug delivery system can be prescribed for each individual. Certainly, with the development of DDSs and the advent of more drug delivery vehicles in the future, CSC-targeted DDSs would make greater progress.

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Author contributions

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Conflicts of interest

The authors have no conflicts of interest to declare.

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