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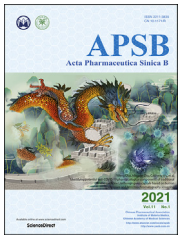

REVIEW

Recent advances in drug delivery systems for targeting cancer stem cells

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KEY WORDS

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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; CMP, carbonate-mannose modified PEI; 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$_{2000}$-DSPE, dequlinium and carboxyl polyethylene glycol-distearoylphosphatidylethanolamine; 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; HIF1α, 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-propylenecarbonate-*graft*-dodecanol-*graft*-cationic ligands); MSNs, mesoporous silica nanoparticles; Nav, navitoclax; ncRNA, non-coding RNAs; NF-κB, nuclear factor-kappa B; PBAEs, poly(β-aminoester); PDT, photodynamic therapy; PEG-*b*-PLA, poly(ethylene glycol)-*block*-poly(D,L-lactide); PEG-PCD, poly(ethylene glycol)-*block*-poly(2-methyl-2-carboxyl-propylene carbonate-*graft*-dodecanol); PEG-PLA, poly(ethylene glycol)-*b*-poly(D,L-lactide); PLGA, poly(ethylene glycol)-poly(D,L-lactide-*co*-glycolide); PTX, paclitaxel; PU-PEI, polyurethane-short branch-polyethylenimine; Sali-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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Niche;
Biomarker;
Cellular level;
Molecular level

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.

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[1,2]. 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[3]. Therefore, tumor recurrence and metastasis have remained a challenge[4]. With research, the cancer stem cells (CSCs) theory[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[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[7].

Moreover, as postulated by the "seed-soil" theory of organ specificity for tumor metastasis[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"[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.)[10,11]. 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[12]. On the other hand, the niche is one of the most important tumor heterogeneity elements that drive cancer drug resistance[13]. 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[14]. 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[15]. 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[16–19]. 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[20–22]. 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[23]. 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[11]) and their associated specific mechanisms[24–31] (Figs. 3–7). Furthermore, it will discuss the

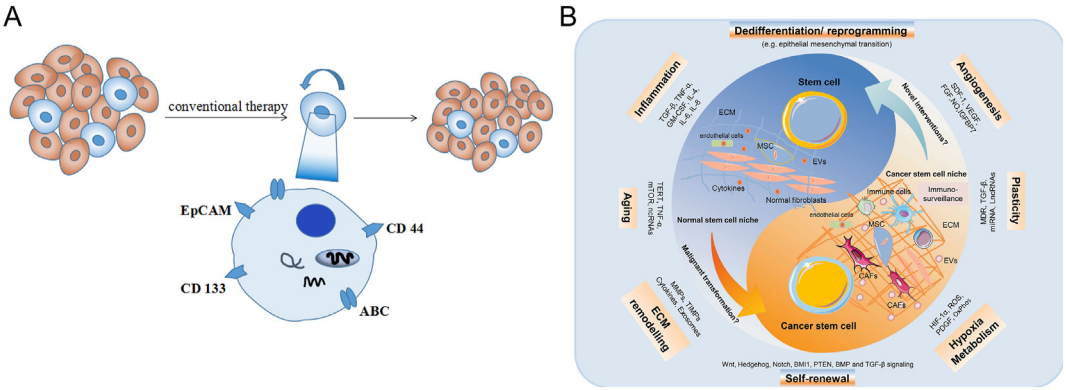

Figure 1 Schematic illustration of multiple unique characteristics of cancer stem cells (CSCs) (A) CSCs possess the ability of self-renewal and differentiation, and express high levels of drug efflux pumps and boosting special biomarkers (B) CSCs reside in complex niche. Reprinted with the permission from Ref. 6. Copyright © 2018 The Authors. Published by Elsevier Ltd.

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[32] or through stem gene activation[10]. 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[33,34]. In this complex niche, whether in a primary or secondary niche, CSCs exhibit significant phenotypic and functional heterogeneity, and their progenies exhibit different plasticity[35]. This may be another Darwinian selection during tumor progression[36]. 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[37]. 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[38]. In addition, some components within the niche can serve as a stimulus and mediate a smart targeting delivery[23]. 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[39,40], 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[41]. 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.[42], 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[43]. 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. Chiou et al.[44] 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.[45] 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)[46] pathways. Furthermore, increasing evidence indicates that these pathways can interact with other cellular signaling pathways such as the NF-κB, MAPK, PI3K, and EGF

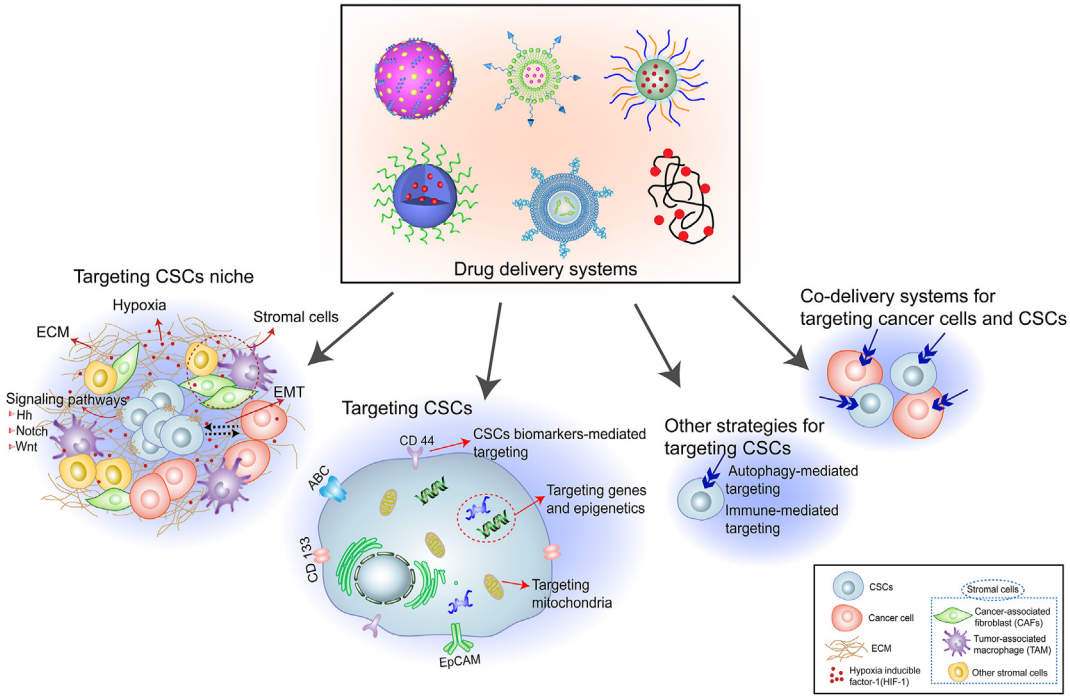

Figure 2 Emerging cancer stem cells (CSCs)-targeted drug delivery systems for efficient cancer therapy following the main line of CSC occurrence and development process.

pathways[47], and it can be said that these intricate signaling 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.[48] 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.[49] developed U0126-loaded NPs (NP_{U0126}) 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 NP_{U0126} 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 NP_{U0126} significantly inhibited CSC self-renewal and interfered with CSC stemness. In a bid to further improve CSC targeting, Miller-Kleinhenz et al.[50] developed a dual targeting iWNT-ATF_{24}-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-ATF_{24}-IONP-DOX simultaneously and effectively down-regulated the WNT/β-catenin pathway, uPAR expression, and CSC-associated biomarkers, resulting in inhibited cell invasion and proliferation of $CD44^{high}/CD24^{low}$ cancer stem-like cell population. Similar results were obtained in an orthotropic chemo-resistant breast cancer PDX model *in vivo*. Table 1[48–61] summarizes more DDSs and carriers that have been targeted at the CSC signaling pathways.

In addition, there were other DDSs or carriers designed for targeting ECM and cancer-associated fibroblasts (CAFs) within the niche. Goodman et al.[62] used collagenase-coated polystyrene NPs to lead collagenase into the CSC niche, thereby degrading the ECM. Chen et al.[63] reported the development of a novel tumor stroma-targeted nanoliposome 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[38]. 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)[64].

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[65]. There have been quite a lot of biomarkers described, such as CD44, CD133, and epithelial cell adhesion molecule (EpCAM)[66]. 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[13,38,67]. In this respect, targeting CSCs based on their specific biomarkers is unquestionably a rational choice, and correspondingly, a great amount of biomarkers-

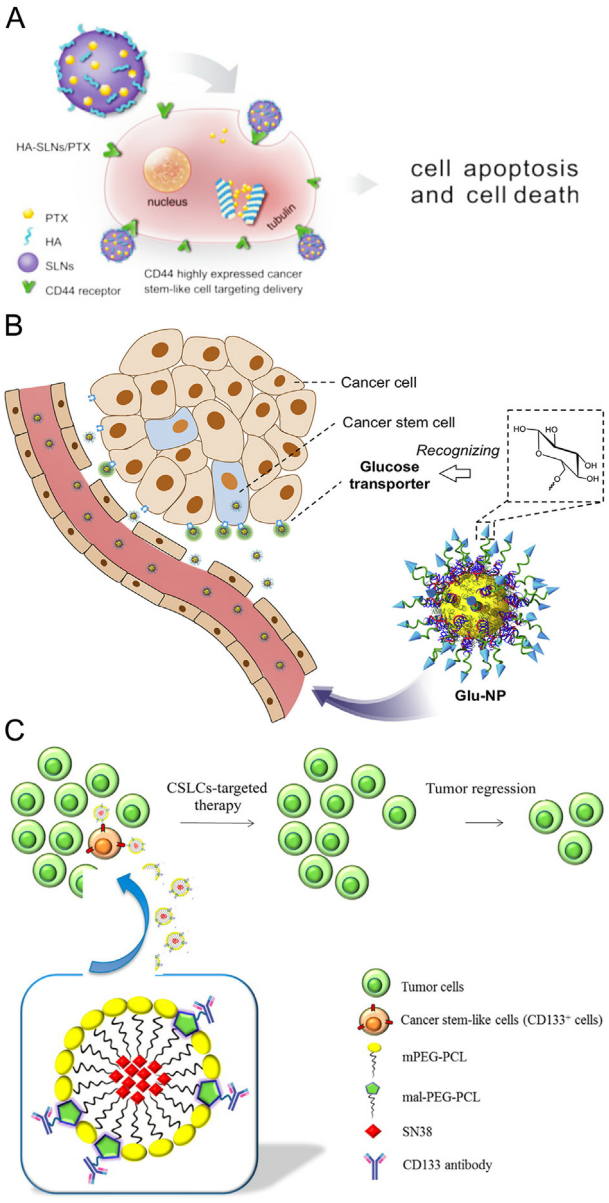

Figure 3 Schematic illustration of cancer stem cell (CSC) biomarkers-mediated delivery systems (A) Schematic illustration of HA-SLNs/PTX delivering PTX to CSCs through HA-mediated mechanism (B) Schematic illustration of Glu-NPs for the targeted delivery of siPLK1 through selective recognition of the glucose ligand to GLUT1. Reprinted with the permission from Ref. 25. Copyright © 2019 Elsevier B.V. (C) Schematic illustration of CD133Ab-NPs to effectively deliver SN38 through receptor-mediated endocytosis. Reprinted with the permission from Ref. 26. Copyright © 2016 American Chemical Society.

mediated delivery carriers and systems have been developed and explored. Table 2[24–26,68-90] 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[91]. In respect of this, Shen et al.[24] 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 (IC_{50}) (11.13 ± 1.62 μg/mL) was achieved by HA-SLNs/PTX, whereas the IC_{50} 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.[25] 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.[26] fabricated PEG-PCL-based NPs conjugated with anti-CD133 antibody to effectively deliver SN-38, a topoisomerase inhibitor, to target CD133-positive (CD133$^+$) 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.[80] proposed the use of a mesoporous silica NP-based nucleus-targeted nanodelivery system to deliver tirapazamine (TPZ) (CD133/TAT/TPZ-Fe_3O_4@$mSiO_2$ NPs), an anticancer drug, to hypoxic CSCs. First, as TPZ plays its role mainly in the nucleus, the constructed CD133/TAT/TPZ-Fe_3O_4@mSiO2 NPs positively targeted CSCs *via* anti-CD133-CD133 receptor interaction. 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 Fe_3O_4 NPs core generated heat to enhance chemosensitivity. Further exploration showed that the inhibition of the expression of hypoxia-inducible factor 1-alpha (HIF1α) by CD133/TAT/TPZ-Fe_3O_4@mSiO2 NPs, which in turn attenuated the hypoxia-signaling pathway, led to the elimination of CSCs. Miyano et al.[92] 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 $\alpha_v\beta_5$ 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 $\alpha_v\beta_5$ 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.

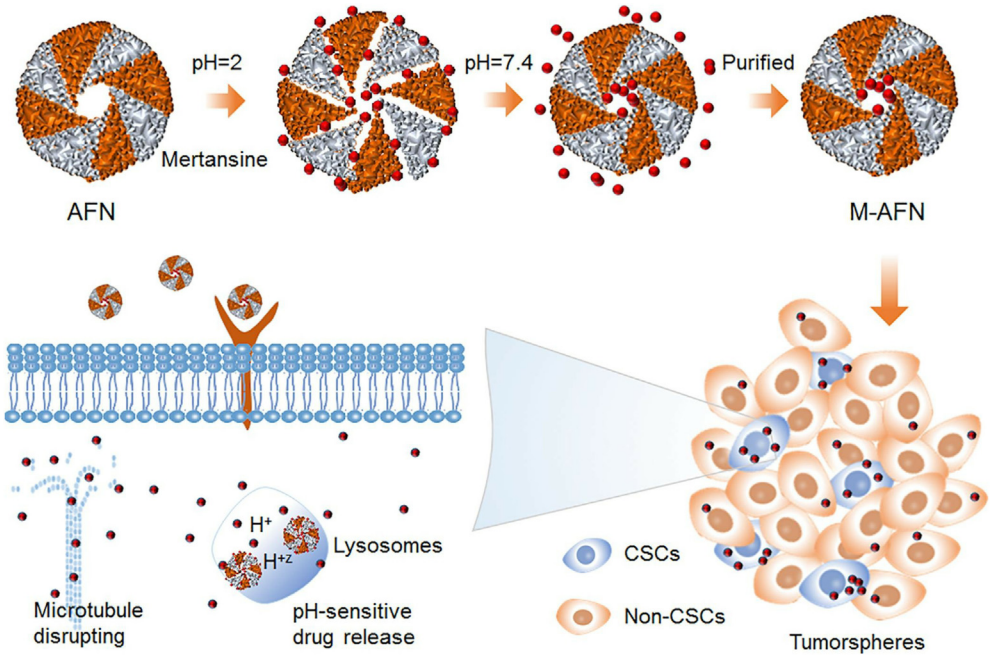

Figure 4 Schematic illustration of mertansine-apoferritin (M-AFN) preferentially taken up by cancer stem cells (CSCs)-enriched tumorspheres. Reprinted with the permission from Ref. 27. Copyright © 2018 Elsevier B.V.

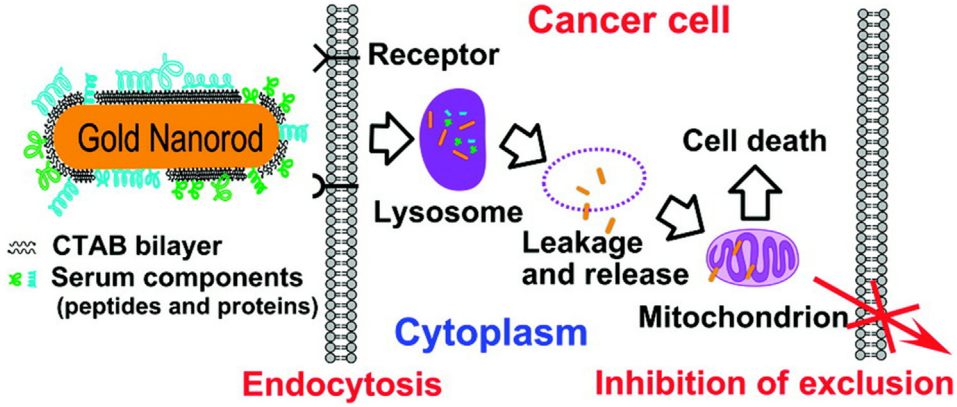

Figure 5 Schematic illustration of mitochondria-targeted delivery. Reprinted with the permission from Ref. 28. Copyright © 2010 American Chemical Society.

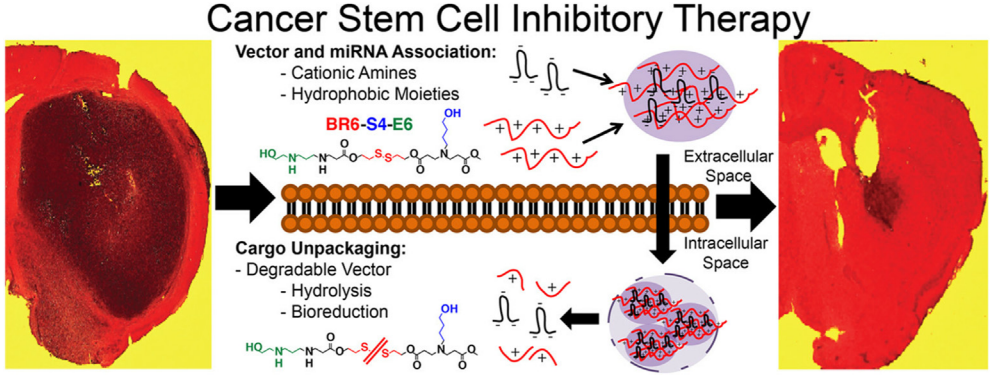

Figure 6 Schematic illustration of R646 nano-miRs targeting multiplexed cancer stem cells-regulating miRNAs. Reprinted with the permission from Ref. 29. Copyright © 2018 American Chemical Society.

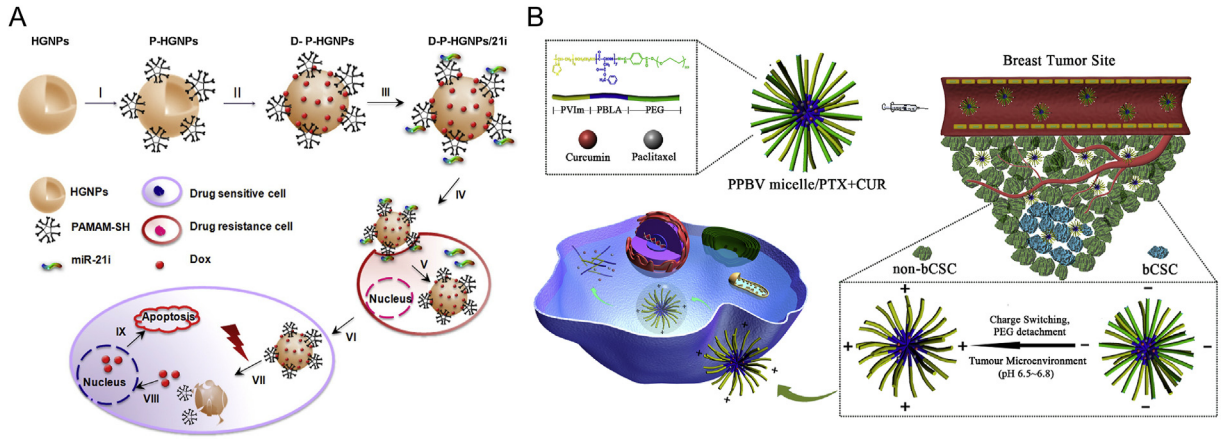

Figure 7 Schematic illustration of co-delivery system for simultaneously eliminating cancer stem cells (CSCs) and non-CSCs (A) HGNPs for co-delivering miR-21i and DOX. Reprinted with the permission from Ref. 30. Copyright © 2016 Elsevier B.V. (B) PPBV micelles for co-delivering paclitaxel and curcumin. Reprinted with the permission from Ref. 31. Copyright © 2017 Elsevier Ltd.

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 ATF_{24} peptides	Dual receptor targeted iWnt-ATF_{24}-IONP	MDA-MB-231	50
STAT3	Niclosamide	Active targeting A15-SLNs	OSCC cells	51
WNT	CGX1321	Liposome	HEK293, LoVo	52
Proteasome	BTZ	PEG-*b*-PLA NPs	MDA-MB-468, HCC1937	53
PI3K/AKT	PEG-coated GNP and cold plasma		A549, T98G	54
P53/P21 dependent manner	QAuNP		H-357-PEMT	55
TGF-β	LY364947 and si*Plk1*	Cationic lipid-assisted polymeric NPs	MDA-MB-231	56
PI3K	siAKT2	Triblock structured PM based on the combination of PEI with Pluronic® amphiphilic copolymers	MCF-7, MDA-MB-231	57
AKT independent pathway	CDF	Liposome	CCL-23, UM-SCC-1	58
NOTCH	DAPT	MSN-PEI-GA_{org} NPs	MDA-MB-231	59
FER	FER siRNA	LMWP	MDA-MB-231	60
β-Catenin	LincRNA-P21	Ad-lnc-P21-MRE	HCT116	61

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[93]. Qiao et al.[88] 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[27,94–98]). Tan et al.[27] used apoferritin, a material that could be preferentially recognized and internalized by CSCs[99], 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.[94] 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.[95] 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 2 Targeting CSCs by biomarkers-mediated carriers and drug delivery systems.

Surface biomarker	Therapeutic agent	Carrier and feature	Cell line	Ref.
CD44	PTX	HA-SLNs	B16F10	24
	DOX	Dual-aptamer-conjugated liposome	MCF-7, HepG2	68
	Anti-luciferase siRNA	HA-conjugated lipoplexes	A549	69
	DOX	HA-SS-MP micelles	HCT116	70
	Doxil	Liposome	C-26	71
	SAL	SWNT-CHI-HA	AGS	72
	8-HQ	HA-MSS	MCF-7	73
	GEM	HA-conjugated liposomes	MCF-7	74
	ATRA	HA-eNPs	B16F10	75
	CDF	HA-SMA	MiaPaCa-2, AsPC-1	76
	DOX and ICG	C60 fullerene-silica NPs decorated with HA	MDA-MB-231, MCF-7	77
	CPT and DOX	HAC-PFP-DC	PC-3, MDA-MB-231	78
GLUT1	siPLK1	Glu-NPs	MDA-MB-231	25
ITGA5	NCTD	RGD-LPH NPs	MDA-MB-231, LM2, SUM159	79
CD133	SN-38	mPEG-PCL/mal-PEG-PCL NP	HT-29, SW620, HCT116	26
	TPZ	CD133/TAT/TPZ-Fe_3O_4 @mSiO 2	MCF-7	80
	Gef	Nanomicelles with CD133 aptamers	A549, A431	81
EpCAM	DOX	EpCAM Apt-DOX	HT29	82
ABCG2	EPI	Lipid MBs conjugated with anti-ABCG2 Ab + UTMD	RPMI 8226	83
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-HA-PEG-PLGA NPs	4T1	88
CD44 and integrin $\alpha_v\beta_3$	mTRAIL plasmid	RRPHC ternary complexes	B16F10	89
EGFR and CD133	Sali	CESP	Saos-2, MG-63	90

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.[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.[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[100]. 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[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[102]. Recently, several scientists reported that chloroquine (CQ) could inhibit autophagy, and nano DDSs together with CQ have enormous potential for improved cancer treatment[103–105]. Sun et al.[106] encapsulated DOX and DTXL with CQ in poly(ethylene glycol)-*block*-poly(D,L-lactide) (PEG-*b*-PLA) NPs (denoted as NP_{CQ}/NP_{DOX} and NP_{CQ}/NP_{DTXL}, 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 NP_{CQ}/NP_{DOX} and NP_{CQ}/NP_{DTXL} could be properly and uniformly dispersed in aqueous solution. Sorted $ALDH^{hi}$ MDA-MB-231 cell tests indicated that the administration of NP_{CQ}/NP_{DOX} and NP_{CQ}/NP_{DTXL} particularly optimized the level of $ALDH^{hi}$ MDA-MB-231 cells and succeeded in hindering mammosphere formation and tumor enlargement. Encouragingly, tumor growth was remarkably impeded, and NP_{CQ}/NP_{DOX} and NP_{CQ}/NP_{DTXL} 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[107,108]. In light of less sensitivity of CSCs to conventional methods and the

Table 3 Well-designed delivery systems transforming conventional chemotherapeutic agents into CSC killers.

Drug	Well-designed delivery systems	Cell line	Ref.
Mertansine	AFN	4T1	27
DOX	DOX-Hyd@AuNPs	MDA-MB-231, BT-474, MCF-7	94
Epirubicin	Nanodiamonds	LT2-MYC	95
DOX	Polymeric micelles	MDA-MB-231, MDA-MB-468	96
DOX	PPC-Hyd-DOX-DA NPs	MDA-MB-231, SK-3rd	97
DOX and shABCG2	MSN-SS-PEI	Hep3B	98

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.[109] 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 Dashti and his partners[110]. In addition to CSC-based vaccines, numerous monoclonal antibodies targeted directly at CSC surface biomarkers are involved in immunotherapy. Bourseau-Guilmain et al.[111] 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[112,113]. 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[114], and so do the corresponding drugs that can effectively target mitochondrial functions[115]. 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 [28].

Wang et al.[28] 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.[116] 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[117]. 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.[118] developed a targeting berberine liposome, which was generated using a mitochondria-tropic functional material conjugated by dequlinium and carboxyl polyethylene glycol-distearoylphosphatidylethanolamine (DQA-PEG$_{2000}$-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[119]. In fact, with the development of the concept of epigenetics over the past several years[120,121], increasing evidence indicates that epigenetics also play profound and ubiquitous roles in the pathogenesis of cancer[122]. 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)[123]. 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[124,125]. 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[126,127]. Therefore, several suitable delivery carriers and systems have been developed. Table 4[29,128–141] summarizes recent DDSs for targeting CSC genes and epigenetics.

Ke et al.[128] 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 carbonate-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.[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[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[143]. Lopez-Bertoni et al.[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 *in vivo* experiments showed that sphere-forming capacity and tumor burden were significantly reduced and tumor cell death was markedly increased. Mittal et al.[130] co-delivered gemcitabine and miRNA-205 (miR-205), an miRNA that is up-regulated in both bulk tumor cells and CSCs resistant to gemcitabine-conjugated poly(ethylene glycol)-*block*-poly(2-methyl-2-carboxyl-propylene-carbonate-*graft*-dodecanol-*graft*-cationicligands) (mPEG-*b*-PCC-*g*-GEM-*g*-DC-*g*-CAT). Jang et al.[131] introduced PLI/miR-34a complex into a nanovesicle (NVs/miR-34a) in order to knock-down CD44 overexpression in a gastric cancer xenograft model.

However, even though numerous miRNA delivery systems have been developed, the challenges associated with the direct delivery of oligonucleotide-based miRNAs, such as higher off-target delivery and poor *in vivo* stability, have remained. Therefore, it is imperative to reverse the situation by delivering small molecules with similar functions to corresponding miRNAs instead of miRNAs. Wen et al.[132] 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.

6. DDSs for combination therapy

It has been demonstrated that combinatorial 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[144,145]. Recently, many researchers have reported successful development of co-delivery systems for combination therapy (Table 5[30,31,73,146–175]; Fig. 7[30,31]). Zhao et al.[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.[147] adopted a micellar NP (MNP) to co-deliver a platinum (IV) prodrug and NOTCH1-targeting siRNA ($^{Pt(IV)}$MNP/siNotch1) to treat CSCs-harboring HCC. The results showed that $^{Pt(IV)}$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, $^{Pt(IV)}$MNP/siNotch1 significantly reduced the stemness of SMMC7721 cells,

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-2^{R}, CAPAN-1^{R}	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
AnxA2	shAnxA2	CLG	H1650	134
Bmi1	Bmi1siR	NPC	HepG2	135
miR145	miR145	PU-PEI	GBM-CD133^{+} cells	136
Let-7 miRNA and CDK4	let-7 miRNA and CDK4-specific siRNA	PEGylated liposome conjugated with herceptin	SK-BR-3	137
Let-7a	Let-7a	PU-PEI-NLS	AA-isolated SP^{+} cells	138
miR-200c	miR-200c	Cationic SLN	MCF-7	139
miR-let-7b	miR-let-7b	UTMD	A2780	140
P70^{S6K}	P70^{S6K} siRNA	G_6 dendriplexes	SKOV-3,HEYA8	141

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 tumors, 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 aforementioned DDSs and carriers effectively ameliorated the shortcomings 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 pharmacokinetic characteristics, inhibitory effects, release performances, and the drug−drug interactions of the different drugs within the carriers, 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 proliferate, move to another suitable niche with CSC niche-like features, and subsequently reestablish their own CSC niche. The existing CSC niche is conducive to the survival of CSCs, and the

Table 5 Co-delivery systems for combination therapy of cancer cells and CSCs.

Co-delivered payload		Carrier and feature	Cell line	Ref.
Drug against cancer cells	Drug against CSCs			
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	iTEP NPs	4T1	146
Platinum (IV)	si*Notch1*	MNP	Hep3B, SMMC7721	147
DOX and SN38		PEG−DOX/SN38 NPs	MCF7	148
DOX	ATRT	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/hTERT	157
Paclitaxel	Artemether	Liposome modified with MAN-TPGS$_{1000}$ and DQA-PEG$_{2000}$-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	OVCAR8, NCI/ADR-RES	175

appropriate 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.

References

1. WHO. *cancer* [EB/OL]. 2019. Available from: https://www.who.int/news-room/fact-sheets/detail/cancer≥.
2. *WHO report on cancer: setting priorities, investing wisely and providing care for all*. Geneva: World Health Organization; 2020. Available from: https://creativecommons.org/licenses/by-nc-sa/3.0/igo.
3. Hidalgo M. Pancreatic cancer. *N Engl J Med* 2010;**362**:1605−17.
4. Chang JC. Cancer stem cells: role in tumor growth, recurrence, metastasis, and treatment resistance. *Medicine* 2016;**95**:S20−5.
5. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001;**414**:105−11.
6. Zhao Y, Dong QZ, Li JH, Zhang KL, Qin J, Zhao JG, et al. Targeting cancer stem cells and their niche: perspectives for future therapeutic targets and strategies. *Semin Canc Biol* 2018;**53**:139−55.
7. Chen K, Huang YH, Chen JL. Understanding and targeting cancer stem cells: therapeutic implications and challenges. *Acta Pharmacol Sin* 2013;**34**:732−40.
8. Paget S. The distribution of secondary growths in cancer of the breast. *Lancet* 1889;**133**:571−3.
9. Lau EY, Ho NP, Lee TK. Cancer stem cells and their microenvironment: biology and therapeutic implications. *Stem Cell Int* 2017;**2017**:3714190.
10. Plaks V, Kong N, Werb Z. The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells?. *Cell stem cell* 2015;**16**:225−38.
11. Adjei IM, Blanka S. Modulation of the tumor microenvironment for cancer treatment: a biomaterials approach. *J Funct Biomater* 2015;**6**:81−103.
12. Kirtane AR, Kalscheuer SM, Panyam J. Exploiting nanotechnology to overcome tumor drug resistance: challenges and opportunities. *Adv Drug Deliv Rev* 2013;**65**:1731−47.
13. Iyer AK, Singh A, Ganta S, Amiji MM. Role of integrated cancer nanomedicine in overcoming drug resistance. *Adv Drug Deliv Rev* 2013;**65**:1784−802.
14. Pattabiraman DR, Weinberg RA. Tackling the cancer stem cells—what challenges do they pose?. *Nat Rev Drug Discov* 2014;**13**:497−512.
15. Putzer BM, Solanki M, Herchenroder O. Advances in cancer stem cell targeting: how to strike the evil at its root. *Adv Drug Deliv Rev* 2017;**120**:89−107.
16. Bidram E, Esmaeili Y, Ranji-Burachaloo H, Al-Zaubai N, Zarrabi A, Stewart A, et al. A concise review on cancer treatment methods and delivery systems. *J Drug Deliv Sci Technol* 2019;**54**:101350.
17. Jimenez-Lopez J, El-Hammadi MM, Ortiz R, Cayero-Otero MD, Cabeza L, Perazzoli G, et al. A novel nanoformulation of PLGA with high non-ionic surfactant content improves *in vitro* and *in vivo* PTX activity against lung cancer. *Pharmacol Res* 2019;**141**:451−65.
18. van Uden DJP, van Maaren MC, Strobbe LJA, Bult P, Stam MR, van der Hoeven JJ, et al. Better survival after surgery of the primary tumor in stage IV inflammatory breast cancer. *Surg Oncol* 2020;**33**:43−50.
19. Gelatti ACZ, Drilon A, Santini FC. Optimizing the sequencing of tyrosine kinase inhibitors (TKIs) in epidermal growth factor receptor (EGFR) mutation-positive non-small cell lung cancer (NSCLC). *Lung Canc* 2019;**137**:113−22.
20. Hermawan A, Putri H. Current report of natural product development against breast cancer stem cells. *Int J Biochem Cell Biol* 2018;**104**:114−32.
21. Cianciosi D, Varela-Lopez A, Forbes-Hernandez TY, Gasparrini M, Afrin S, Reboredo-Rodriguez P, et al. Targeting molecular pathways in cancer stem cells by natural bioactive compounds. *Pharmacol Res* 2018;**135**:150−65.
22. Lv JF, Shim JS. Existing drugs and their application in drug discovery targeting cancer stem cells. *Arch Pharm Res (Seoul)* 2015;**38**:1617−26.
23. Dianat-Moghadam H, Heidarifard M, Jahanban-Esfahlan R, Panahi Y, Hamishehkar H, Pouremamali F, et al. Cancer stem cells-emanated therapy resistance: implications for liposomal drug delivery systems. *J Contr Release* 2018;**288**:62−83.
24. Shen HX, Shi SJ, Zhang ZR, Gong TG, Sun X. Coating solid lipid nanoparticles with hyaluronic acid enhances antitumor activity against melanoma stem-like cells. *Theranostics* 2015;**5**:755−71.
25. Yi Y, Kim HJ, Zheng M, Mi P, Naito M, Kim BS, et al. Glucose-linked sub-50-nm unimer polyion complex-assembled gold nanoparticles for targeted siRNA delivery to glucose transporter 1-overexpressing breast cancer stem-like cells. *J Contr Release* 2019;**295**:268−77.
26. Ning ST, Lee SY, Wei MF, Peng CL, Lin SY, Tsai MH, et al. Targeting colorectal cancer stem-like cells with anti-CD133 antibody-conjugated SN-38 nanoparticles. *ACS Appl Mater Interfaces* 2016;**8**:17793−804.
27. Tan T, Wang YQ, Wang H, Cao HQ, Wang ZW, Wang J, et al. Apoferritin nanocages loading mertansine enable effective eradiation of cancer stem-like cells *in vitro*. *Int J Pharm* 2018;**553**:201−9.
28. Wang LM, Liu Y, Li W, Jiang XM, Ji YL, Wu XC, et al. Selective targeting of gold nanorods at the mitochondria of cancer cells: implications for cancer therapy. *Nano Lett* 2011;**11**:772−80.

29. Lopez-Bertoni H, Kozielski KL, Rui Y, Lal B, Vaughan H, Wilson DR, et al. Bioreducible polymeric nanoparticles containing multiplexed cancer stem cell regulating miRNAs inhibit glioblastoma growth and prolong survival. *Nano Lett* 2018;**18**:4086−94.
30. Ren Y, Wang RR, Gao LZ, Li K, Zhou X, Guo H, et al. Sequential co-delivery of miR-21 inhibitor followed by burst release doxorubicin using NIR-responsive hollow gold nanoparticle to enhance anticancer efficacy. *J Contr Release* 2016;**228**:74−86.
31. Yang Z, Sun N, Cheng R, Zhao CY, Liu Z, Li X, et al. pH multistage responsive micellar system with charge-switch and PEG layer detachment for co-delivery of paclitaxel and curcumin to synergistically eliminate breast cancer stem cells. *Biomaterials* 2017;**147**:53−67.
32. Najafi M, Mortezaee K, Ahadi R. Cancer stem cell (a) symmetry & plasticity: tumorigenesis and therapy relevance. *Life Sci* 2019;**231**:116520.
33. Borovski T, De Sousa EMF, Vermeulen L, Medema JP. Cancer stem cell niche: the place to be. *Can Res* 2011;**71**:634−9.
34. Hiratsuka S, Watanabe A, Aburatani H, Maru Y. Tumour-mediated upregulation of chemoattractants and recruitment of myeloid cells predetermines lung metastasis. *Nat Cell Biol* 2006;**8**:1369−75.
35. Tang DG. Understanding cancer stem cell heterogeneity and plasticity. *Cell Res* 2012;**22**:457−72.
36. Boesch M, Sopper S, Zeimet AG, Reimer D, Gastl G, Ludewig B, et al. Heterogeneity of cancer stem cells: rationale for targeting the stem cell niche. *Biochim Biophys Acta* 2016;**1866**:276−89.
37. Malanchi I, Santamaria-Martinez A, Susanto E, Peng H, Lehr HA, Delaloye JF, et al. Interactions between cancer stem cells and their niche govern metastatic colonization. *Nature* 2011;**481**:85−9.
38. Garcia-Mazas C, Csaba N, Garcia-Fuentes M. Biomaterials to suppress cancer stem cells and disrupt their tumoral niche. *Int J Pharm* 2017;**523**:490−505.
39. Das B, Tsuchida R, Malkin D, Koren G, Baruchel S, Yeger H. Hypoxia enhances tumor stemness by increasing the invasive and tumorigenic side population fraction. *Stem Cell* 2008;**26**:1818−30.
40. Dimou A, Syrigos KN, Saif MW. Overcoming the stromal barrier: technologies to optimize drug delivery in pancreatic cancer. *Ther Adv Med Oncol* 2012;**4**:271−9.
41. Usacheva M, Swaminathan SK, Kirtane AR, Panyam J. Enhanced photodynamic therapy and effective elimination of cancer stem cells using surfactant-polymer nanoparticles. *Mol Pharm* 2014;**11**:3186−95.
42. Wang H, Li J, Wang YQ, Gong X, Xu XX, Wang JY, et al. Nanoparticles-mediated reoxygenation strategy relieves tumor hypoxia for enhanced cancer therapy. *J Contr Release* 2020;**319**:25−45.
43. Bradford GB, Williams B, Rossi R, Bertoncello I. Quiescence, cycling, and turnover in the primitive hematopoietic stem cell compartment. *Exp Hematol* 1997;**25**:445−53.
44. Chiou GY, Cherng JY, Hsu HS, Wang ML, Tsai CM, Lu KH, et al. Cationic polyurethanes-short branch PEI-mediated delivery of mir 145 inhibited epithelial-mesenchymal transdifferentiation and cancer stem-like properties and in lung adenocarcinoma. *J Contr Release* 2012;**159**:240−50.
45. Ahmad A, Mondal SK, Mukhopadhyay D, Banerjee R, Alkharfy KM. Development of liposomal formulation for delivering anticancer drug to breast cancer stem-cell-like cells and its pharmacokinetics in an animal model. *Mol Pharm* 2016;**13**:1081−8.
46. Shukla S, Meeran SM. Epigenetics of cancer stem cells: pathways and therapeutics. *Biochim Biophys Acta* 2014;**1840**:3494−502.
47. Takebe N, Miele L, Harris PJ, Jeong W, Bando H, Kahn M, et al. Targeting Notch, Hedgehog, and Wnt pathways in cancer stem cells: clinical update. *Nat Rev Clin Oncol* 2015;**12**:445−64.
48. Karandish F, Froberg J, Borowicz P, Wilkinson JC, Choi Y, Mallik S. Peptide-targeted, stimuli-responsive polymersomes for delivering a cancer stemness inhibitor to cancer stem cell microtumors. *Colloids Surf B Biointerfaces* 2018;**163**:225−35.
49. Liu Y, Wang X, Sun CY, Wang J. Delivery of mitogen-activated protein kinase inhibitor for hepatocellular carcinoma stem cell therapy. *ACS Appl Mater Interfaces* 2015;**7**:1012−20.
50. Miller-Kleinhenz J, Guo XX, Qian WP, Zhou HY, Bozeman EN, Zhu L, et al. Dual-targeting Wnt and uPA receptors using peptide conjugated ultra-small nanoparticle drug carriers inhibited cancer stem-cell phenotype in chemo-resistant breast cancer. *Biomaterials* 2018;**152**:47−62.
51. Pindiprolu SH, Pindiprolu S. CD133 receptor mediated delivery of STAT3 inhibitor for simultaneous elimination of cancer cells and cancer stem cells in oral squamous cell carcinoma. *Med Hypotheses* 2019;**129**:109241.
52. Li C, Liang YY, Cao J, Zhang N, Wei XH, Tu MQ, et al. The delivery of a Wnt pathway inhibitor toward CSCs requires stable liposome encapsulation and delayed drug release in tumor tissues. *Mol Ther* 2019;**27**:1558−67.
53. Shen S, Du XJ, Liu J, Sun R, Zhu YH, Wang J. Delivery of bortezomib with nanoparticles for basal-like triple-negative breast cancer therapy. *J Contr Release* 2015;**208**:14−24.
54. Kaushik NK, Kaushik N, Yoo KC, Uddin N, Kim JS, Lee SJ, et al. Low doses of PEG-coated gold nanoparticles sensitize solid tumors to cold plasma by blocking the PI3K/AKT-driven signaling axis to suppress cellular transformation by inhibiting growth and EMT. *Biomaterials* 2016;**87**:118−30.
55. Satapathy SR, Nayak A, Siddharth S, Das S, Nayak D, Kundu CN. Metallic gold and bioactive quinacrine hybrid nanoparticles inhibit oral cancer stem cell and angiogenesis by deregulating inflammatory cytokines in p53 dependent manner. *Nanomedicine* 2018;**14**:883−96.
56. Zuo ZQ, Chen KG, Yu XY, Zhao G, Shen S, Cao ZT, et al. Promoting tumor penetration of nanoparticles for cancer stem cell therapy by TGF-beta signaling pathway inhibition. *Biomaterials* 2016;**82**:48−59.
57. Rafael D, Gener P, Andrade F, Seras-Franzoso J, Montero S, Fernandez Y, et al. AKT2 siRNA delivery with amphiphilic-based polymeric micelles show efficacy against cancer stem cells. *Drug Deliv* 2018;**25**:961−72.
58. Basak SK, Zinabadi A, Wu AW, Venkatesan N, Duarte1 VM, Kang JJ, et al. Liposome encapsulated curcumin-difluorinated (CDF) inhibits the growth of cisplatin resistant head and neck cancer stem cells. *Oncotarget* 2015;**6**:18504−17.
59. Mamaeva V, Niemi R, Beck M, Ozliseli E, Desai D, Landor S, et al. Inhibiting Notch activity in breast cancer stem cells by glucose functionalized nanoparticles carrying gamma-secretase Inhibitors. *Mol Ther* 2016;**24**:926−36.
60. Suh JS, Lee HJ, Nam H, Jo BS, Lee DW, Kim JH, et al. Control of cancer stem cell like population by intracellular target identification followed by the treatment with peptide-siRNA complex. *Biochem Biophys Res Commun* 2017;**491**:827−33.
61. Wang J, Lei ZJ, Guo Y, Wang T, Qin ZY, Xiao HL, et al. miRNA-regulated delivery of lincRNA-p21 suppresses β-catenin signaling and tumorigenicity of colorectal cancer stem cells. *Oncotarget* 2015;**6**:37852−70.
62. Goodman TT, Olive PL, Pun SH. Increased nanoparticle penetration in collagenase-treated multicellular spheroids. *Int J Nanomed* 2007;**2**:265−74.
63. Chen BL, Dai WB, Mei D, Liu TZ, Li SX, He B, et al. Comprehensively priming the tumor microenvironment by cancer-associated fibroblast-targeted liposomes for combined therapy with cancer cell-targeted chemotherapeutic drug delivery system. *J Contr Release* 2016;**241**:68−80.
64. Zhou Y, Zhou WX, Chen XL, Wang QB, Li C, Chen QJ, et al. Bone marrow mesenchymal stem cells-derived exosomes for penetrating and targeted chemotherapy of pancreatic cancer. *Acta Pharm Sin B* 2020;**10**:1563−75.
65. Woodward WA, Sulman EP. Cancer stem cells:markers or biomarkers?. *Canc Metastasis Rev* 2008;**27**:459−70.
66. Mokhtarzadeh A, Hassanpour S, Vahid ZF, Hejazi M, Hashemi M, Ranjbari J, et al. Nano-delivery system targeting to cancer stem cell cluster of differentiation biomarkers. *J Contr Release* 2017;**266**:166−86.
67. Shen S, Xia JX, Wang J. Nanomedicine-mediated cancer stem cell therapy. *Biomaterials* 2016;**74**:1−18.

68. Kim DM, Kim M, Park HB, Kim KS, Kim DE. Anti-MUC1−CD44 dual-aptamer-conjugated liposomes for cotargeting breast cancer cells and cancer stem cells. *ACS Appl. Bio Mater* 2019;**2**:4622−33.
69. Leite Nascimento T, Hillaireau H, Vergnaud J, Rivano M, Delomenie C, Courilleau D, et al. Hyaluronic acid-conjugated lipoplexes for targeted delivery of siRNA in a murine metastatic lung cancer model. *Int J Pharm* 2016;**514**:103−11.
70. Debele TA, Yu LY, Yang CS, Shen YA, Lo CL. pH- and GSH-Sensitive hyaluronic acid-MP conjugate micelles for intracellular delivery of doxorubicin to colon cancer cells and cancer stem cells. *Biomacromolecules* 2018;**19**:3725−37.
71. Arabi L, Badiee A, Mosaffa F, Jaafari MR. Targeting CD44 expressing cancer cells with anti-CD44 monoclonal antibody improves cellular uptake and antitumor efficacy of liposomal doxorubicin. *J Contr Release* 2015;**220**:275−86.
72. Yao HJ, Zhang YG, Sun L, Liu Y. The effect of hyaluronic acid functionalized carbon nanotubes loaded with salinomycin on gastric cancer stem cells. *Biomaterials* 2014;**35**:9208−23.
73. Wang D, Huang JB, Wang XX, Yu Y, Zhang H, Chen Y, et al. The eradication of breast cancer cells and stem cells by 8-hydroxyquinoline-loaded hyaluronan modified mesoporous silica nanoparticle-supported lipid bilayers containing docetaxel. *Biomaterials* 2013;**34**:7662−73.
74. Han NK, Shin DH, Kim JS, Weon KY, Jang CY, Kim JS. Hyaluronan-conjugated liposomes encapsulating gemcitabine for breast cancer stem cells. *Int J Nanomed* 2016;**11**:1413−25.
75. Li YY, Shi SJ, Ming Y, Wang LL, Li CW, Luo MH, et al. Specific cancer stem cell-therapy by albumin nanoparticles functionalized with CD44-mediated targeting. *J Nanobiotechnol* 2018;**16**:99.
76. Kesharwani P, Banerjee S, Padhye S, Sarkar FH, Iyer AK. Hyaluronic acid engineered nanomicelles loaded with 3,4-difluorobenzylidene curcumin for targeted killing of $CD44^+$ stem-like pancreatic cancer cells. *Biomacromolecules* 2015;**16**:3042−53.
77. Wang H, Agarwal P, Zhao ST, Yu JH, Lu XB, He XM. Combined cancer therapy with hyaluronan-decorated fullerene-silica multifunctional nanoparticles to target cancer stem-like cells. *Biomaterials* 2016;**97**:62−74.
78. Wang H, Agarwal P, Zhao ST, Xu RX, Yu JH, Lu XB, et al. Hyaluronic acid-decorated dual responsive nanoparticles of Pluronic F127, PLGA, and chitosan for targeted co-delivery of doxorubicin and irinotecan to eliminate cancer stem-like cells. *Biomaterials* 2015;**72**:74−89.
79. Li YF, Xiao YJ, Lin HP, Reichel D, Bae Y, Lee EY, et al. *In vivo* beta-catenin attenuation by the integrin alpha5-targeting nano-delivery strategy suppresses triple negative breast cancer stemness and metastasis. *Biomaterials* 2019;**188**:160−72.
80. Li HJ, Yan WX, Suo XM, Peng HT, Yang XJ, Li ZH, et al. Nucleus-targeted nano delivery system eradicates cancer stem cells by combined thermotherapy and hypoxia-activated chemotherapy. *Biomaterials* 2019;**200**:1−14.
81. Huang XL, Huang JS, Leng DW, Yang S, Yao Q, Sun J, et al. Gefitinib-loaded DSPE-PEG_{2000} nanomicelles with CD133 aptamers target lung cancer stem cells. *World J Surg Oncol* 2017;**15**:167.
82. Xiang DX, Shigdar S, Bean AG, Bruce M, Yang WR, Mathesh M, et al. Transforming doxorubicin into a cancer stem cell killer *via* EpCAM aptamer-mediated delivery. *Theranostics* 2017;**7**:4071−86.
83. Shi FF, Li M, Wu SY, Yang F, Di W, Pan M, et al. Enhancing the anti-multiple myeloma efficiency in a cancer stem cell xenograft model by conjugating the ABCG2 antibody with microbubbles for a targeted delivery of ultrasound mediated epirubicin. *Biochem Pharmacol* 2017;**132**:18−28.
84. Liang SJ, Li C, Zhang CL, Chen YS, Xu L, Bao CC, et al. CD44v6 monoclonal antibody-conjugated gold nanostars for targeted photoacoustic imaging and plasmonic photothermal therapy of gastric cancer stem-like cells. *Theranostics* 2015;**5**:970−84.
85. Li J, Xu WQ, Yuan XL, Chen HW, Song H, Wang BQ, et al. Polymer−lipid hybrid anti-HER2 nanoparticles for targeted salinomycin delivery to HER2-positive breast cancer stem cells and cancer cells. *Int J Nanomed* 2017;**12**:6909−21.
86. Zeng YB, Yu ZC, He YN, Zhang T, Du LB, Dong YM, et al. Salinomycin-loaded lipid-polymer nanoparticles with anti-CD20 aptamers selectively suppress human $CD20^+$ melanoma stem cells. *Acta Pharmacol Sin* 2018;**39**:261−74.
87. Sun TM, Wang Y, Wang YC, Xu JB, Zhao X, Vangveravong S, et al. Using SV119-gold nanocage conjugates to eradicate cancer stem cells through a combination of photothermal and chemo therapies. *Adv Healthc Mater* 2014;**3**:1283−91.
88. Qiao SP, Zhao YF, Geng S, Li Y, Hou XL, Liu Y, et al. A novel double-targeted nondrug delivery system for targeting cancer stem cells. *Int J Nanomed* 2016;**11**:6667−78.
89. Li L, Song LJ, Yang X, Li X, Wu YZ, He T, et al. Multifunctional "core-shell" nanoparticles-based gene delivery for treatment of aggressive melanoma. *Biomaterials* 2016;**111**:124−37.
90. Chen FY, Zeng YB, Qi XX, Chen YC, Ge Z, Jiang ZX, et al. Targeted salinomycin delivery with EGFR and CD133 aptamers based dual-ligand lipid-polymer nanoparticles to both osteosarcoma cells and cancer stem cells. *Nanomedicine* 2018;**14**:2115−27.
91. Heldin P, Kolliopoulos C, Lin CY, Heldin CH. Involvement of hyaluronan and CD44 in cancer and viral infections. *Cell Signal* 2020;**65**:109427.
92. Miyano K, Cabral H, Miura Y, Matsumoto Y, Mochida Y, Kinoh H, et al. cRGD peptide installation on cisplatin-loaded nanomedicines enhances efficacy against locally advanced head and neck squamous cell carcinoma bearing cancer stem-like cells. *J Contr Release* 2017;**261**:275−86.
93. Ahmad G, Amiji MM. Cancer stem cell-targeted therapeutics and delivery strategies. *Expet Opin Drug Deliv* 2017;**14**:997−1008.
94. Sun TM, Wang YC, Wang F, Du JZ, Mao CQ, Sun CY, et al. Cancer stem cell therapy using doxorubicin conjugated to gold nanoparticles *via* hydrazone bonds. *Biomaterials* 2014;**35**:836−45.
95. Wang X, Low XC, Hou WX, Abdullah LN, Toh TB, Rashid MMA, et al. Epirubicin-adsorbed nanodiamonds kill chemoresistant hepatic cancer stem cells. *ACS Nano* 2014;**8**:12151−66.
96. Zhao Y, Alakhova DY, Zhao XS, Band V, Batrakova EV, Kabanov AV. Eradication of cancer stem cells in triple negative breast cancer using doxorubicin/pluronic polymeric micelles. *Nanomedicine* 2020;**24**:102124.
97. Du JZ, Du XJ, Mao CQ, Wang J. Tailor-made dual pH-sensitive polymer–doxorubicin nanoparticles for efficient anticancer drug delivery. *J Am Chem Soc* 2011;**133**:17560−3.
98. Chen ZZ, Zhu PP, Zhang YS, Liu YT, He YL, Zhang LF, et al. Enhanced sensitivity of cancer stem cells to chemotherapy using functionalized mesoporous silica nanoparticles. *Mol Pharm* 2016;**13**:2749−59.
99. Schonberg David L, Miller Tyler E, Wu Q, Flavahan William A, Das Nupur K, Hale James S, et al. Preferential iron trafficking characterizes glioblastoma stem-like cells. *Canc Cell* 2015;**28**:441−55.
100. Galluzzi L, Pietrocola F, Levine B, Kroemer G. Metabolic control of autophagy. *Cell* 2014;**159**:1263−76.
101. Lei YY, Zhang D, Yu J, Dong H, Zhang JW, Yang SM. Targeting autophagy in cancer stem cells as an anticancer therapy. *Canc Lett* 2017;**393**:33−9.
102. Nazio F, Bordi M, Cianfanelli V, Locatelli F, Cecconi F. Autophagy and cancer stem cells: molecular mechanisms and therapeutic applications. *Cell Death Differ* 2019;**26**:690−702.
103. Liang DH, Choi DS, Ensor JE, Kaipparettu BA, Bass BL, Chang JC. The autophagy inhibitor chloroquine targets cancer stem cells in triple negative breast cancer by inducing mitochondrial damage and impairing DNA break repair. *Canc Lett* 2016;**376**:249−58.
104. Pelt J, Busatto S, Ferrari M, Thompson EA, Mody K, Wolfram J. Chloroquine and nanoparticle drug delivery: a promising combination. *Pharmacol Ther* 2018;**191**:43−9.
105. Joshi P, Chakraborti S, Ramirez-Vick JE, Ansari ZA, Shanker V, Chakrabarti P, et al. The anticancer activity of chloroquine-gold

nanoparticles against MCF-7 breast cancer cells. *Colloids Surf B Biointerfaces* 2012;**95**:195−200.
106. Sun R, Shen S, Zhang YJ, Xu CF, Cao ZT, Wen LP, et al. Nanoparticle-facilitated autophagy inhibition promotes the efficacy of chemotherapeutics against breast cancer stem cells. *Biomaterials* 2016;**103**:44−55.
107. Fakhrejahani F, Tomita Y, Maj-Hes A, Trepel JB, De Santis M, Apolo AB. Immunotherapies for bladder cancer: a new hope. *Curr Opin Urol* 2015;**25**:586−96.
108. Liu SY, Wu YL. An immunological storm for cancer therapy: 2018 Nobel Prize in Physiology or Medicine. *Sci Bull* 2018;**63**:1608−10.
109. El-Ashmawy NE, El-Zamarany EA, Salem ML, Khedr EG, Ibrahim AO. A new strategy for enhancing antitumor immune response using dendritic cells loaded with chemo-resistant cancer stem-like cells in experimental mice model. *Mol Immunol* 2019;**111**:106−17.
110. Dashti A, Ebrahimi M, Hadjati J, Memarnejadian A, Moazzeni SM. Dendritic cell based immunotherapy using tumor stem cells mediates potent antitumor immune responses. *Canc Lett* 2016;**374**:175−85.
111. Bourseau-Guilmain E, Béjaud J, Griveau A, Lautram N, Hindré F, Weyland M, et al. Development and characterization of immunonanocarriers targeting the cancer stem cell marker AC133. *Int J Pharm* 2012;**423**:93−101.
112. Folmes CDL, Ma H, Mitalipov S, Terzic A. Mitochondria in pluripotent stem cells: stemness regulators and disease targets. *Curr Opin Genet Dev* 2016;**38**:1−7.
113. Xu XL, Duan SL, Yi F, Ocampo A, Liu GH, Izpisua Belmonte Juan C. Mitochondrial regulation in pluripotent stem cells. *Cell Metabol* 2013;**18**:325−32.
114. Loureiro R, Mesquita KA, Magalhães-Novais S, Oliveira PJ, Vega-Naredo I. Mitochondrial biology in cancer stem cells. *Semin Canc Biol* 2017;**47**:18−28.
115. Skoda J, Borankova K, Jansson PJ, Huang MLH, Veselska R, Richardson DR. Pharmacological targeting of mitochondria in cancer stem cells: an ancient organelle at the crossroad of novel anti-cancer therapies. *Pharmacol Res* 2019;**139**:298−313.
116. Zhang L, Yao HJ, Yu Y, Zhang Y, Li RJ, Ju RJ, et al. Mitochondrial targeting liposomes incorporating daunorubicin and quinacrine for treatment of relapsed breast cancer arising from cancer stem cells. *Biomaterials* 2012;**33**:565−82.
117. Mignotte B, Vayssiere JL. Mitochondria and apoptosis. *Eur J Biochem* 1998;**252**:1−15.
118. Ma X, Zhou J, Zhang CX, Li XY, Li N, Ju RJ, et al. Modulation of drug-resistant membrane and apoptosis proteins of breast cancer stem cells by targeting berberine liposomes. *Biomaterials* 2013;**34**:4452−65.
119. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. *Nature* 2009;**458**:719−24.
120. Mendell Joshua T, Olson Eric N. MicroRNAs in stress signaling and human disease. *Cell* 2012;**148**:1172−87.
121. Dawson MA, Kouzarides T. Cancer epigenetics: from mechanism to therapy. *Cell* 2012;**150**:12−27.
122. Nebbioso A, Tambaro FP, Dell'Aversana C, Altucci L. Cancer epigenetics: moving forward. *PLoS Genet* 2018;**14**:e1007362.
123. Dumitrescu RG, Verma M. *Cancer epigenetics*. New York: Humana Press; 2012.
124. Sakamoto N, Honma R, Sekino Y, Goto K, Sentani K, Ishikawa A, et al. Non-coding RNAs are promising targets for stem cell-based cancer therapy. *Noncoding RNA Res* 2017;**2**:83−7.
125. Mimeault M, Batra SK. Altered gene products involved in the malignant reprogramming of cancer stem/progenitor cells and multitargeted therapies. *Mol Aspect Med* 2014;**39**:3−32.
126. Shim MS, Kwon YJ. Efficient and targeted delivery of siRNA *in vivo*. *FEBS J* 2010;**277**:4814−27.
127. Mirza Z, Karim S. Nanoparticles-based drug delivery and gene therapy for breast cancer: recent advancements and future challenges. *Semin Canc Biol* 2019. Available from: https://doi.org/10.1016/j.semcancer.2019.10.020.
128. Ke XY, Yang C, Cheng W, Yang YY. Delivery of NF-κB shRNA using carbamate-mannose modified PEI for eliminating cancer stem cells. *Nanomedicine* 2018;**14**:405−14.
129. Andey T, Bora-Singhal N, Chellappan SP, Singh M. Cationic lipoplexes for treatment of cancer stem cell-derived murine lung tumors. *Nanomedicine* 2019;**18**:31−43.
130. Mittal A, Chitkara D, Behrman SW, Mahato RI. Efficacy of gemcitabine conjugated and miRNA-205 complexed micelles for treatment of advanced pancreatic cancer. *Biomaterials* 2014;**35**:7077−87.
131. Jang E, Kim E, Son HY, Lim EK, Lee H, Choi Y, et al. Nanovesicle-mediated systemic delivery of microRNA-34a for CD44 overexpressing gastric cancer stem cell therapy. *Biomaterials* 2016;**105**:12−24.
132. Wen D, Peng Y, Lin F, Singh RK, Mahato RI. Micellar delivery of miR-34a modulator rubone and paclitaxel in resistant prostate cancer. *Can Res* 2017;**77**:3244−54.
133. Verma RK, Yu W, Singh SP, Shankar S, Srivastava RK. Anthothecol-encapsulated PLGA nanoparticles inhibit pancreatic cancer stem cell growth by modulating sonic hedgehog pathway. *Nanomedicine* 2015;**11**:2061−70.
134. Andey T, SrujanMarepally, Patel A, Jackson T, Sarkar S, O'Connell M, et al. Cationic lipid guided short-hairpin RNA interference of annexin A2 attenuates tumor growth and metastasis in amouse lung cancer stem cell model. *J Contr Release* 2014;**184**:67−78.
135. Yang T, Chen YY, Zhao PX, Xue HY, You J, Li B, et al. Enhancing the therapeutic effect *via* elimination of hepatocellular carcinoma stem cells using Bmi1 siRNA delivered by cationic cisplatin nanocapsules. *Nanomedicine* 2018;**14**:2009−21.
136. Yang YP, Chien Y, Chiou GY, Cherng JY, Wang ML, Lo WL, et al. Inhibition of cancer stem cell-like properties and reduced chemoradioresistance of glioblastoma using microRNA145 with cationic polyurethane-short branch PEI. *Biomaterials* 2012;**33**:1462−76.
137. Shin JH, Shin DH, Kim J-S. Let-7 miRNA and CDK4 siRNA co-encapsulated in Herceptin-conjugated liposome for breast cancer stem cells. *Asian J Pharm Sci* 2020;**15**:472−81.
138. Yang MY, Chen MT, Huang PI, Wang CY, Chang YC, Yang YP, et al. Nuclear localization signal-enhanced polyurethane-short branch polyethylenimine-mediated delivery of Let-7a inhibited cancer stem-like properties by targeting the 3′-UTR of HMGA2 in anaplastic astrocytoma. *Cell Transplant* 2015;**24**:1431−50.
139. Liu JW, Meng TT, Ming Y, Wen LJ, Cheng BL, Liu N, et al. MicroRNA-200c delivered by solid lipid nanoparticles enhances the effect of paclitaxel on breast cancer stem cell. *Int J Nanomed* 2016;**11**:6713−25.
140. Yang CP, Li BC, Yu JS, Yang F, Cai K, Chen ZY. Ultrasound microbubbles mediated miR-let-7b delivery into $CD133^{+}$ ovarian cancer stem cells. *Biosci Rep* 2018;**38**. BSR20180922.
141. Ma J, Kala S, Yung SS, Chan TM, Cao Y, Jiang YF, et al. Blocking stemness and metastatic properties of ovarian cancer cells by targeting $p70^{S6K}$ with dendrimer nanovector-based siRNA delivery. *Mol Ther* 2018;**26**:70−83.
142. Caldas C, Brenton JD. Sizing up miRNAs as cancer genes. *Nat Med* 2005;**11**:712−4.
143. Garofalo M, Croce CM. Role of microRNAs in maintaining cancer stem cells. *Adv Drug Deliv Rev* 2015;**81**:53−61.
144. Oak PS, Kopp F, Thakur C, Ellwart JW, Rapp UR, Ullrich A, et al. Combinatorial treatment of mammospheres with trastuzumab and salinomycin efficiently targets HER2-positive cancer cells and cancer stem cells. *Int J Canc* 2012;**131**:2808−19.
145. Han L, Shi SJ, Gong T, Zhang ZR, Sun X. Cancer stem cells: therapeutic implications and perspectives in cancer therapy. *Acta Pharm Sin B* 2013;**3**:65−75.
146. Zhao P, Xia GQ, Dong SY, Jiang ZX, Chen MN. An iTEP-salinomycin nanoparticle that specifically and effectively inhibits metastases of 4T1 orthotopic breast tumors. *Biomaterials* 2016;**93**:1−9.

147. Shen S, Sun CY, Du XJ, Li HJ, Liu Y, Xia JX, et al. Co-delivery of platinum drug and siNotch1 with micelleplex for enhanced hepatocellular carcinoma therapy. *Biomaterials* 2015;**70**:71−83.
148. Sun N, Zhao CY, Cheng R, Liu ZR, Li X, Lu AX, et al. Cargo-free nanomedicine with pH sensitivity for codelivery of DOX conjugated prodrug with SN38 to synergistically eradicate breast cancer stem cells. *Mol Pharm* 2018;**15**:3343−55.
149. Sun R, Liu Y, Li SY, Shen S, Du XJ, Xu CF, et al. Co-delivery of all-trans-retinoic acid and doxorubicin for cancer therapy with synergistic inhibition of cancer stem cells. *Biomaterials* 2015;**37**:405−14.
150. Kim YJ, Liu YR, Li S, Rohrs J, Zhang R, Zhang XY, et al. Co-eradication of breast cancer cells and cancer stem cells by cross-linked multilamellar liposomes enhances tumor treatment. *Mol Pharm* 2015;**12**:2811−22.
151. Yang RN, Mondal G, Wen D, Mahato RI. Combination therapy of paclitaxel and cyclopamine polymer-drug conjugates to treat advanced prostate cancer. *Nanomedicine* 2017;**13**:391−401.
152. Al Faraj A, Shaik AS, Ratemi E, Halwani R. Combination of drug-conjugated SWCNT nanocarriers for efficient therapy of cancer stem cells in a breast cancer animal model. *J Contr Release* 2016;**225**:240−51.
153. Tsai PH, Wang ML, Chang JH, Yarmishyn AA, Nhi Nguyen PN, Chen W, et al. Dual delivery of HNF4α and cisplatin by mesoporous silica nanoparticles inhibits cancer pluripotency and tumorigenicity in hepatoma-derived CD133-expressing stem cells. *ACS Appl Mater Interfaces* 2019;**11**:19808−18.
154. Uz M, Kalaga M, Pothuraju R, Ju J, Junker WM, Batra SK, et al. Dual delivery nanoscale device for miR-345 and gemcitabine co-delivery to treat pancreatic cancer. *J Contr Release* 2019;**294**:237−46.
155. Shi SJ, Han L, Deng L, Zhang YL, Shen HX, Gong T, et al. Dual drugs (microRNA-34a and paclitaxel)-loaded functional solid lipid nanoparticles for synergistic cancer cell suppression. *J Contr Release* 2014;**194**:228−37.
156. Zhang JJ, Kinoh H, Hespel L, Liu XY, Quader S, Martin J, et al. Effective treatment of drug resistant recurrent breast tumors harboring cancer stem-like cells by staurosporine/epirubicin co-loaded polymeric micelles. *J Contr Release* 2017;**264**:127−35.
157. Zhou Y, Yang JY, Rhim JS, Kopecek J. HPMA copolymer-based combination therapy toxic to both prostate cancer stem/progenitor cells and differentiated cells induces durable anti-tumor effects. *J Contr Release* 2013;**172**:946−53.
158. Li XY, Zhao Y, Sun MG, Shi JF, Ju RJ, Zhang CX, et al. Multifunctional liposomes loaded with paclitaxel and artemether for treatment of invasive brain glioma. *Biomaterials* 2014;**35**:5591−604.
159. Kinoh H, Miura Y, Chida T, Liu XY, Mizuno K, Fukushima S, et al. Nanomedicines eradicating cancer stem-like cells *in vivo* by pH-triggered intracellular cooperative action of loaded drugs. *ACS Nano* 2016;**10**:5643−55.
160. Zhang Y, Zhang H, Wang XQ, Wang JC, Zhang X, Zhang Q. The eradication of breast cancer and cancer stem cells using octreotide modified paclitaxel active targeting micelles and salinomycin passive targeting micelles. *Biomaterials* 2012;**33**:679−91.
161. Wu PY, Liu Q, Wang Q, Qian HQ, Yu LX, Liu BR, et al. Novel silk fibroin nanoparticles incorporated silk fibroin hydrogel for inhibition of cancer stem cells and tumor growth. *Int J Nanomed* 2018;**13**:5405−18.
162. Huang WT, Larsson M, Lee YC, Liu DM, Chiou GY. Dual drug-loaded biofunctionalized amphiphilic chitosan nanoparticles: enhanced synergy between cisplatin and demethoxycurcumin against multidrug-resistant stem-like lung cancer cells. *Eur J Pharm Biopharm* 2016;**109**:165−73.
163. Yang Q, Zhang T, Wang CL, Jiao J, Li J, Deng YH. Coencapsulation of epirubicin and metformin in PEGylated liposomes inhibits the recurrence of murine sarcoma S180 existing $CD133^+$ cancer stem-like cells. *Eur J Pharm Biopharm* 2014;**88**:737−45.
164. Ke XY, Lin Ng VW, Gao SJ, Tong YW, Hedrick JL, Yang YY. Co-delivery of thioridazine and doxorubicin using polymeric micelles for targeting both cancer cells and cancer stem cells. *Biomaterials* 2014;**35**:1096−108.
165. Muntimadugu E, Kumar R, Saladi S, Rafeeqi TA, Khan W. CD44 targeted chemotherapy for co-eradication of breast cancer stem cells and cancer cells using polymeric nanoparticles of salinomycin and paclitaxel. *Colloids Surf B Biointerfaces* 2016;**143**:532−46.
166. Lin F, Wen D, Wang XF, Mahato RI. Dual responsive micelles capable of modulating miRNA-34a to combat taxane resistance in prostate cancer. *Biomaterials* 2019;**192**:95−108.
167. Fonseca NA, Rodrigues AS, Rodrigues-Santos P, Alves V, Gregorio AC, Valerio-Fernandes A, et al. Nucleolin overexpression in breast cancer cell sub-populations with different stem-like phenotype enables targeted intracellular delivery of synergistic drug combination. *Biomaterials* 2015;**69**:76−88.
168. Guo X, Guo N, Zhao JW, Cai YL. Active targeting co-delivery system based on hollow mesoporous silica nanoparticles for anti-tumor therapy in ovarian cancer stem-like cells. *Oncol Rep* 2017;**38**:1442−50.
169. Li WJ, Yan RC, Liu Y, He CC, Zhang XJ, Lu Y, et al. Co-delivery of Bmi1 small interfering RNA with ursolic acid by folate receptor-targeted cationic liposomes enhances anti-tumor activity of ursolic acid *in vitro* and *in vivo*. *Drug Deliv* 2019;**26**:794−802.
170. Li L, Cui DJ, Ye LM, Li Y, Zhu LY, Yang LQ, et al. Codelivery of salinomycin and docetaxel using poly(D,L-lactic-*co*-glycolic acid)-poly(ethylene glycol) nanoparticles to target both gastric cancer cells and cancer stem cells. *Anti Canc Drugs* 2017;**28**:989−1001.
171. Mahira S, Kommineni N, Husain GM, Khan W. Cabazitaxel and silibinin co-encapsulated cationic liposomes for CD44 targeted delivery: a new insight into nanomedicine based combinational chemotherapy for prostate cancer. *Biomed Pharmacother* 2019;**110**:803−17.
172. Liang DS, Liu J, Peng TX, Peng H, Guo F, Zhong HJ. Vitamin E-based redox-sensitive salinomycin prodrug-nanosystem with paclitaxel loaded for cancer targeted and combined chemotherapy. *Colloids Surf B Biointerfaces* 2018;**172**:506−16.
173. Hu KL, Zhou HG, Liu Y, Liu Z, Liu J, Tang JL, et al. Hyaluronic acid functional amphipathic and redox-responsive polymer particles for the co-delivery of doxorubicin and cyclopamine to eradicate breast cancer cells and cancer stem cells. *Nanoscale* 2015;**7**:8607−18.
174. Lang TQ, Liu YR, Zheng Z, Ran W, Zhai YH, Yin Q, et al. Cocktail strategy based on spatio-temporally controlled nano device improves therapy of breast cancer. *Adv Mater* 2019;**31**:e1806202.
175. Zhang XY, Liu YR, Kim YJ, Mac J, Zhuang R, Wang P. Co-delivery of carboplatin and paclitaxel *via* cross-linked multilamellar liposomes for ovarian cancer treatment. *RSC Adv* 2017;**7**:19685−93.