

# Cancer stem cell theory: therapeutic implications for nanomedicine

Ke Wang<sup>1</sup>  
Xianguo Wu<sup>2</sup>  
Jianwei Wang<sup>3</sup>  
Jian Huang<sup>1,3</sup>

<sup>1</sup>Cancer Institute (Key Laboratory of Cancer Prevention and Intervention, National Ministry of Education; Provincial Key Laboratory of Molecular Biology in Medical Sciences), <sup>2</sup>Department of Clinical Laboratory, <sup>3</sup>Department of Oncology, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, People's Republic of China

**Abstract:** Evidence continues to accumulate showing that tumors contain a minority population of cells responsible for tumor initiation, growth, and recurrence. These are termed “cancer stem cells” (CSCs). Functional assays have identified the self-renewal and tumor-initiation capabilities of CSCs. Moreover, recent studies have revealed that these CSCs is responsible for chemotherapy resistance within a tumor. Several mechanisms of chemoresistance have been proposed, including increased Wnt/ $\beta$ -catenin and Notch signaling, as well as high expression levels of adenosine triphosphate-binding cassette transporters, an active DNA repair capacity, and slow rate of self-renewal. Nanoscale drug-delivery systems, which transport therapeutically active molecules, prolong circulation, and improve biodistribution in the body, may allow more effective and specific therapies to address the challenges posed by CSCs. In particular, some nanovehicles are being exploited for selective drug delivery to CSCs and show promising results. In this review, we highlight the mechanisms of drug resistance and the novel strategies using nanoscale drugs to eliminate CSCs.

**Keywords:** drug resistance, drug delivery, chemoresistance, Wnt/ $\beta$ -catenin signaling, Notch signaling

## Introduction

Cancer is becoming more recognized as a heterogeneous disease with hierarchies of cellular populations that demonstrate a range of differentiation phenotypes. The majority of cells in bulk tumors may be non-tumorigenic end cells, and only a small subpopulation of cells within tumors is responsible for tumor initiation, growth, and recurrence. These are called “cancer stem cells” (CSCs).<sup>1,2</sup> CSCs possess both self-renewal and differentiation capabilities.<sup>3</sup> Several signaling pathways are involved in the self-renewal behavior of CSCs, including Wnt/ $\beta$ -catenin, Notch, and hedgehog signaling, which mediate the resistances against radiotherapy and chemotherapy.<sup>4</sup> Despite the moderate success of currently available therapeutic approaches to tumors, they have several limitations. One of the main therapy drawbacks is that there is insufficient elimination of CSCs. Further, frequently there is multiple drug resistance (MDR) with advanced tumors.<sup>5</sup> Surviving CSCs will lead to tumor recurrence. Therefore, attention has been focused on defining new agents and novel therapies for cancer prevention and therapy by eliminating CSCs.

Nanoscale drug-delivery systems for cancer therapeutics are rapidly evolving and may offer an innovative approach to overcome the drug resistances of CSCs. Recently, nanoparticle-based strategies have demonstrated enhanced therapeutic efficacy and reduced adverse side effects, compared with those of classical therapeutic methods.

Correspondence: Jianwei Wang;  
Jian Huang  
Department of Oncology, Second  
Affiliated Hospital, Zhejiang  
University School of Medicine,  
Hangzhou 310009, People's  
Republic of China  
Tel +86 571 87315009  
Fax +86 571 87022776  
Email bljianwei@126.com;  
drhuangjian@zju.edu.cn

Properly designed nanoparticles have the ability to significantly accumulate in tumor tissues by extravasation of nanoparticulates through fenestrated tumor vasculature via either passive or active targeting.<sup>6</sup> Moreover, loaded-drug formulations show excellent tumor cell uptake characteristics, block drug efflux from cancer cells, and reverse the MDR of tumors.<sup>7,8</sup>

To improve the outcome of cancer treatments, we need to comprehend characteristics of CSCs, and propose new strategies to eliminate CSCs based on the available literature.

## CSCs

Despite the ongoing debate over CSCs exist, there is no denying that most cancers are heterogeneous and show functional and phenotypical differences at the cell population level. These observations may result from clonal evolution driven by the differentiation of CSCs or from genetic instability.<sup>9,10</sup> Moreover, CSCs can vary between different patient tumors and can constantly change as the disease progresses. Therefore, for cancer prevention and treatment, we need to identify and characterize these subpopulations. CSCs may display certain properties; CSC subpopulations: can be isolated based on cell surface marker profiles,<sup>11</sup> exhibit increased resistance against conventional radiotherapy and chemotherapy,<sup>12,13</sup> and may initiate tumors at limiting dilutions in animals.<sup>14</sup> These characteristics imply the existence of a distinct fraction of cancer cells that have a self-renewal property and the potential to cause tumors with only a limited number of cells.

CSCs were first observed in acute myeloid leukemia (AML). There is a small fraction of AML cells with a surface marker phenotype of CD34+CD38- that is able to recapitulate the phenotypes of the original patient tumor in immune-deficient mice.<sup>15</sup> Although new studies have revealed additional unexpected heterogeneity of severe combined immunodeficiency leukemia-initiating cells, CD34-, Lin+, CD38+, and CD45RA+ fractions have the capacity to form xenografted tumors. Subsequently, CSCs were demonstrated to exist in solid tumors, including those formed by brain, breast, colon, prostate, pancreatic, lung, liver, melanoma and ovarian cancers (Table 1).<sup>16-31</sup> These cells express markers of stemness and are capable of reproducing the cancer in mouse models. In breast cancer, CD44+CD24- and aldehyde dehydrogenase 1 (ALDH1)+ have been demonstrated to be selective phenotypes that enrich CSCs.<sup>26,32</sup> Recently, the CD133+ cell subpopulation was found to harbor brain CSCs.<sup>33</sup> However, several studies suggest that the glycosylation status of CD133 molecule,

**Table 1** Identification of CSCs using surface markers

Tumor type	Marker(s)	References
Acute myeloid leukemia	CD34+CD38-	15
Brain	CD133+	16,
Breast	CD44+CD24-, ALDH1+	17,25
Colon	CD133+, CD44+EpCAM+, ALDH1+	19,25,26
Prostate	CD44+ $\alpha$ 2 $\beta$ 1high, ALDH+	18,27
Pancreas	CD133+, ESA+CD44+CD24+	20,28
Lung	CD133+, ALDH+	23,29
Liver	CD90+	24
Ovarian	CD133+, CD44+, ALDH1+	22,30,31
Melanoma	ABC5+	21

**Abbreviation:** ALDH, aldehyde dehydrogenase I.

rather than the expression of the CD133 protein itself, appears to be a marker for CSC phenotypes. Additionally, CD44 is often expressed as a variety of isoforms. CD44v is highly expressed in certain cancers and CD44v6 has been targeted for cancer therapy.

CSCs have also been identified by tumorsphere culture.<sup>34</sup> Similar to the forming of mouse embryonic fibroblasts and neurospheres in suspension culture, CSCs may grow in the absence of serum and without attachment to culture plates, whereas differentiated cells fail to survive under the same conditions.<sup>35</sup> Moreover, CSCs are capable of forming subsequent passages of tumorspheres and multi-lineage differentiation. These properties can be used to identify the self-renewal capacity of CSCs for treatment with or without drugs in vitro.

## Self-renewal pathways of CSCs

CSCs produce tumors through self-renewal and differentiation regulated by several signaling pathways (Table 2).<sup>36-42</sup> Understanding the mechanisms of self-renewal in CSCs is of great importance for drug discovery and development. Wnt/ $\beta$ -catenin signaling is one of the key pathways that modulates CSC self-renewal.<sup>43,44</sup> Activation of Wnt-target genes depends on mediation by  $\beta$ -catenin, which enters the nucleus from the cytoplasm, then cooperates with the TCF/LEF transcription factor, eventually resulting in the activation of Wnt-target genes such as *cyclin D1*, *c-Jun*, and *c-Myc*.<sup>44,45</sup> Beta-catenin protein is degraded by the ubiquitin-proteasome pathway through phosphorylation at Ser33/Ser37/Thr41 by GSK3 $\beta$ .<sup>46,47</sup> The Wnt/ $\beta$ -catenin pathway is implicated in the maintenance of CSC self-renewal in leukemia, melanoma, and breast, lung, and liver cancers.<sup>48-52</sup> It has been reported that a high level of  $\beta$ -catenin increases the drug resistance of numerous tumor types,<sup>53</sup> indicating that dysregulation

**Table 2** Signaling pathways involved in CSCs self-renewal

Major signaling pathways	Cancer	References
Wnt/ $\beta$ -catenin	Breast cancer	49
	Liver cancer	51
	AML	48
	Melanoma	50
Hedgehog	Glioblastoma	64
	Breast cancer	39
	Colon cancer	65
	Pancreatic cancer	62
	Leukemia	63
Notch	Breast cancer	57
	Colon cancer	56
	Glioblastoma	55
PTEN/PI3-K/Akt	Leukemia	36
	Breast cancer	37
	Glioblastoma	38
BMI1	Breast cancer	39
	Leukemia	40
	Glioblastoma	41
TGF- $\beta$	Glioblastoma	42

**Abbreviations:** PI3-K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; TGF, transforming growth factor.

of  $\beta$ -catenin plays a crucial role in cancer treatment. If the transcriptional activity of  $\beta$ -catenin can be significantly inhibited, cancer growth will be suppressed. Therefore, it is critical that agents be found that can directly target  $\beta$ -catenin and its downstream molecules.

Notch signaling is another important signaling pathway involved in modulation of CSC self-renewal.<sup>54–57</sup> Four Notch genes (*Notch 1* to 4) have been identified in mammals, which act as transmembrane receptors for Jagged 1 and 2, and Delta-like 1, 3, and 4.<sup>58</sup> The binding of ligands to Notch results in its cleavage by A disintegrin and metalloprotease (ADAM) family members and  $\gamma$ -secretase.<sup>59</sup> The intracellular domain of Notch translocates to the nucleus, where it activates downstream target genes such as *cyclin D1*, *c-Myc*, and *nuclear factor kappa B (NF- $\kappa$ B)*.<sup>60</sup> Recent studies have shown that the Notch pathway is upregulated in CSCs, leading to uncontrolled self-renewal. Specifically, Notch 1 has been reported to cross-talk with Wnt/ $\beta$ -catenin signaling in diverse cellular situations and the interaction between Notch and Wnt/ $\beta$ -catenin pathways suggests that Notch is probably involved in CSC-related tumor recurrence following therapy.

Other pathways, such as the hedgehog-signaling pathway, can also maintain the self-renewal of CSCs.<sup>61–65</sup> The hedgehog pathway has been identified to be involved in CSC self-renewal and tumorigenicity in human breast cancer.<sup>39</sup> Further, a previous report showed that the hedgehog pathway is associated with NF- $\kappa$ B signaling, indicating that sonic hedgehog might be activated by the transcription factor NF- $\kappa$ B.<sup>66</sup>

## CSCs and drug resistance

To explain why chemotherapies ultimately fail to cure cancer, the CSC hypothesis suggests that the therapeutic resistance of CSCs in tumors might be the mechanism. Such CSC characteristics might result from several situations, including high expression levels of adenosine triphosphate-binding cassette (ABC) transporters, resistance against apoptosis, an active DNA repair capacity, and slow rate of self-renewal. Studies of cancer cells have revealed that CSCs commonly express high levels of drug efflux pumps.<sup>67,68</sup> Such drug pumps are responsible for protecting cancer cells from damage by cytotoxic chemotherapies via efflux pumping mechanisms.<sup>69</sup> Therefore, as a result of these biophysical and biological properties, CSCs are rendered resistant against chemotherapeutic agents.

Additional studies have revealed that CSCs extrude the fluorescent dye rhodamine 123 and Hoechst 33342. The cells that efflux Hoechst 33342 can be detected by flow cytometry and are called “side population” (SP) cells.<sup>70</sup> The Hoechst efflux assay has successfully identified SP cells in various solid tissues including breast, pancreatic, and liver.<sup>71–73</sup> Moreover, studies have confirmed that chemoresistant cancer cells contain a higher proportion of SP cells than chemosensitive cells.<sup>74</sup> Recent findings suggest that other transporters, including octreotide (Oct) 1, also contribute to CSC resistance against certain drugs. Maddox et al showed that Oct1 controls multiple stem cell phenotypes in both normal and tumor cells.<sup>75</sup> Elevated Oct1 protein expression correlates with elevated CD24<sup>+</sup>CD44<sup>+</sup> or ALDH<sup>hi</sup> CSC populations in breast cancer tissues. Genes associated with drug efflux pumps, such as *Abcg2*, *Abcb1* and *Abcb4*, are directly regulated by Oct1. Furthermore, Oct1 knockdown specifically decreases the number of SP cells among A549 cells.

In addition to possessing an increased capacity for cytotoxic agent efflux, CSCs are identified by their characteristic slow-cycling and quiescent properties.<sup>76</sup> These cells, also termed “label-retaining” cells, can be purified by functional assays.<sup>77,78</sup> Such a small subset of CSCs mostly remains quiescent in the G0 phase. Over time, CSCs are induced to divide and produce transit-amplifying cells by stimuli. Subsequently, some of these transit-amplifying cells differentiate into new mature cancer cells with a chemoresistant phenotype.<sup>79</sup> These observations have been confirmed in CSCs derived from AML and solid tumors.<sup>80,81</sup> The acquired chemoresistance of cancer, which corresponds with the presence of CSCs, increases greatly after chemotherapy in the clinic. Ultimately, patients at this stage will develop recurrent tumors and fail to be responsive to further treatment by chemotherapy.

In a third model of acquired resistance, drug-resistant variants of CSCs or their close descendants arise, which produce a population of DNA-repairing tumor cells.<sup>82,83</sup> A previous study has revealed that CD133+ cells express > 30-fold higher levels of the DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT) than matched CD133- gliomas.<sup>13</sup> Because of the increased DNA repair capacity, CD133+ cells are more resistant to radiotherapy than CD133- cells. Moreover, patients with high expression levels of MGMT demonstrate significantly reduced median survival times compared with patients with low MGMT expression.<sup>84,85</sup> These results suggest that DNA repair may be a target for the elimination of CSCs to facilitate the survival of patients.

CSCs expressing elevated levels of ALDH1, (a molecular metabolic mediator) show resistance against cytotoxic agents.<sup>86</sup> ALDH1 is a detoxification enzyme involved in catalyzing the oxidation of acetaldehydes produced from ethanol.<sup>87</sup> As a detoxification enzyme, overexpression of ALDH1 in CSCs has implications in the resistances against chemotherapeutic drugs such as cyclophosphamide. Furthermore, high ALDH1 activity in cancer is associated with a poor outcome,<sup>88</sup> suggesting that chemoresistant molecules expressed by CSCs will directly affect patient prognoses.

## Nanomaterial strategies for cancer stem cell therapy

The existence of CSCs has important implications for chemoprevention and treatment of cancer. CSCs are more resistant to treatment than bulk cells, meaning that conventional chemotherapies for cancer often fail. Strategies to address this concern include new approaches and therapeutic agents for the reversal of chemotherapy resistance by targeting CSCs. Nanomedicine offers an innovative approach to overcome these hurdles. The potential of nanomedicine lies in the ability to engineer formulations at the nanometer scale for loading chemotherapeutics or active molecules. In addition, the designed vehicles may sensitize or enhance therapeutic strategies that cater to the unique dynamics of cancer.

The nanovehicles that transport therapeutic drugs and facilitate cellular uptake based on self-assembled supra-molecular differ according to the drug and nano-sized carrier. Their development depends on several key factors that govern interactions with the body, including the size, polarity, numbers and hydrophobic or hydrophilic nature of nanoparticles.<sup>89</sup> Nanovehicles prolong circulation and improve the biodistribution of the incorporated drug, yielding

superior accumulation in tumors via a process known as “enhanced permeability and retention.”<sup>90</sup> The virtue of nanovehicles is that they can be adjusted using molecules without loss of activity. Moreover, nanovehicles are used to encapsulate chemotherapeutics, which can hide unfavorable domains between the drug and the body. Based on these advantages, the objective of nanomedicine is to develop new agents to provide beneficial pharmacological properties for eliminating CSCs.

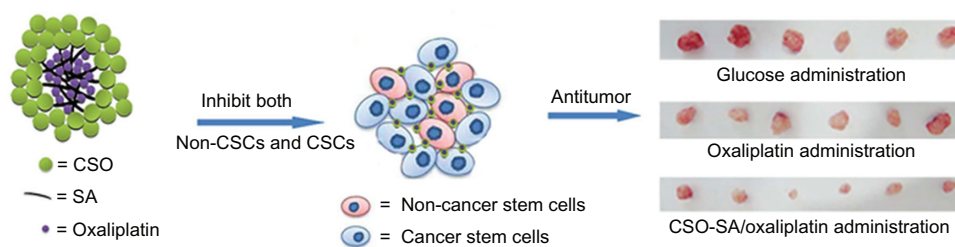
Accordingly, nanomedicine for the targeting of CSCs requires that there be multidisciplinary cooperation to develop new agents as well as accurate interpretation of the data obtained from different disciplines. In particular, to harness the potential of nanobiology and nanomedicine, the properties of CSCs and their role in cancer progression need to be carefully understood. Therefore, novel nanomedicines will need to be created for the development of therapeutic strategies against CSCs.

## Development of nanomedicine for CSCs

### Drug-delivery systems for CSCs

To improve the therapeutic effect on CSCs, nanoscaled drugs have enabled development of many novel strategies to overcome the known shortcomings of many anticancer drugs, such as drug extrusion, low aqueous solubility and stability, and high nonspecific toxicity.<sup>91,92</sup> These nanoparticles include polymeric micelles and non-polymeric systems. Polymeric micelles with a core-shell structure can be formed by the self-aggregation of amphiphilic grafts in water, providing a significant advantage for delivery of cytotoxic agents to cancer.<sup>93,94</sup> Previous studies have demonstrated that cell uptake of drugs is increased using nanovehicles compared with the free drug.<sup>95</sup> For example, we have developed a novel micelle formulation of oxaliplatin encapsulated in a chitosan vesicle (CSO-SA/OXA micelles).<sup>96</sup> These CSO-SA/OXA micelles show an excellent internalization ability that targets the tumor cell nucleus and increases the oxaliplatin concentration in tumor cells, which can reverse the drug resistance of CSCs and effectively eliminate CSCs *in vitro* and *in vivo* (Figure 1). The uptake of nanovehicles may be via endocytosis in which the free drug is internalized into cancer cells by molecular diffusion. Using drug-loaded nanovehicles, an efficient route for drug delivery is penetration of the cell membrane, especially in chemoresistant tumor cells.

In another example, Zhang et al demonstrated the strong therapeutic potential of salinomycin-loaded polyethylene glycol-b-polycaprolactone (PEG-b-PCL) polymeric micelles



**Figure 1** Oxaliplatin-incorporating micelles are effective for the eradication of cancer stem cells (CSCs).

**Note:** The drug-loaded CSO-SA micelles suppress both colorectal CSCs and bulk cancer cells, resulting in enhancement of antitumor efficacy.

**Abbreviations:** CSO, chitosan oligosaccharide; SA, stearic acid.

(M-SAL) and octreotide (Oct)-modified paclitaxel (PTX)-loaded PEG-b-PCL polymeric micelles (Oct-M-PTX) in the treatment of breast CSCs.<sup>97</sup> Oct is an octapeptide analog of endogenous somatostatin and mainly binds to somatostatin receptors (SSTRs) that are overexpressed in many cancers. By coupling Oct, copolymer micelles can enhance their binding to SSTR-positive cancer cells and increase intracellular delivery of drugs. Combinatorial therapy using Oct-M-PTX plus M-SAL may eradicate breast cancer cells together with breast CSCs via receptor-mediated endocytosis.<sup>97</sup>

Similarly, CSC persistence in chronic myeloid leukemia (CML) can also be targeted by vectorized nanocarriers. Bcr-Abl tyrosine-kinase inhibitors (TKIs) are the first-line therapy for most patients with CML. However, imatinib (a TKI) has been shown to be a substrate of ABCG2, and fails to cure end-stage patients. Zhou et al described that the resistance of CML CD34+ and primitive CD34+CD38- cells can be overcome using synthetic low-density lipoprotein (sLDL) particles.<sup>98</sup> sLDL is prepared using a solvent evaporation method involving a mixture consisting of phosphatidylcholine, triolein, cholesterol, and cholesteryl oleate at a molar ratio of 3:2:1:1, respectively. Low-density lipoprotein receptor-specific lipophilic synthetic peptides have been used to target CML cells. The results indicated that Bcr-Abl-positive cell lines show increased and preferential uptake of sLDL compared with Bcr-Abl negative cells.<sup>98</sup>

Nanomedicine has the potential to overcome the known shortcomings of many anticancer agents, including low water-solubility, instability, and nonspecific toxicity. For example, curcumin has been reported to eliminate colorectal CSCs in vitro. However, its application in cancer treatment is limited by high hydrophobicity, instability, and poor pharmacokinetics. Nanoscale drugs offer an innovative approach to overcome such problems. For example, we have prepared a nanotechnology-based curcumin formulation and confirmed that it can self-assemble to form nanoscale micelles in an aqueous medium.<sup>99</sup> Nanoparticles increase the

stability of curcumin by protecting the encapsulated curcumin against hydrolysis and biotransformation. Moreover, the formulation effectively inhibits CSCs and marginally suppresses tumor growth.

### Targeting of signaling pathways in CSCs

Nanovehicles loaded with small molecules to target signaling pathways are another avenue toward the eradication of CSCs. Although surface markers are partly shared with normal stem cells, there are still many differences, including signaling pathway and metabolic alterations in CSCs, which may be exploited for selective targeted delivery of nanoscale drugs. The molecular targeting of deregulated signaling pathways, which may contribute to the chemoresistance of cancer, is currently under concerted investigation. The potential pathways include Wnt/ $\beta$ -catenin, hedgehog, and Notch signaling.<sup>100,101</sup> Zhou et al recently designed an N-(2-hydroxypropyl)methacrylamide (HPMA)-based delivery system for delivery of the hedgehog-signaling inhibitor cyclopamine that is a selective macromolecular therapeutic against CSCs.<sup>102</sup> However, the clinical use of cyclopamine is restricted by its high hydrophobicity and systemic toxicity. A HPMA copolymer has been synthesized by reversible addition-fragmentation chain transfer copolymerization of HPMA and 3-(N-methacryloyl-glycylphenylalanyl-leucylglycyl)-thiazolidine-2-thione (MA-GFLG-TT), followed by conjugation of cyclopamine to glycylphenylalanyl-leucylglycyl side chains. The HPMA copolymer-cyclopamine conjugate binds to cells via the smoothed (SMO) membrane receptor. The authors reported that the HPMA copolymer-cyclopamine conjugate shows a selective inhibitory effect on prostate CSCs in comparison with that on bulk cancer cells.

In another example, the application of  $\gamma$ -secretase inhibitors (GSIs) in cancer treatment to block Notch signaling is limited by their high hydrophobicity and side effects. Mamaeva et al developed mesoporous silica nanoparticles (MSNPs) as vehicles for targeted delivery of GSIs to block

Notch signaling.<sup>103</sup> The folate receptor is overexpressed in many tumors, therefore, a GSI is encapsulated in MSNPs, and folate is covalently conjugated to the outside to target folate receptor-enriched cancer cells. The average size of synthesized MSNPs is 200 to 350 nm. GSI-loaded MSNPs efficiently target and block Notch activity, inhibit tumor growth, and CSC functions in vivo. These biocompatible and biodegradable MSNPs provide a novel platform for efficient small-molecule drug delivery for the development of refined Notch therapy.

Targeting of CSC regulatory pathways by RNA interference (RNAi) has been reported using nanodelivery systems to treat cancers. Lo et al developed nanodelivery of double-stranded DNA (dsDNA) encoding siRNA to efficiently downregulate the activity of EZH2 and Oct4 associated with CSC properties, which directly led to an anticancer effect.<sup>104</sup> By conjugating nuclear localization signal peptides, the efficacy of dsDNA encoding siRNA against EZH2 or Oct4 is enhanced because of the facilitated nuclear delivery. Treatment of head and neck squamous cancer cell xenografts with this formulation remarkably represses CSCs and enhances radiosensitivity, which may involve the Wnt pathway.

In the treatment of glioblastoma (GBM), promising therapeutic approaches include miR145 incorporated with polyurethane-short branch polyethylenimine (PU-PEI) to block key signal transduction pathways, which has been found to effectively mediate downregulation of Oct4 and Sox2 by targeting the Oct4 and Sox2 3' untranslated regions in GBM CD133+ cells.<sup>104</sup> Moreover, real-time polymerase chain reaction analysis has shown that the expression of other stemness genes, such as *Nanog*, *c-Myc*, and the oncogene *Bmi-1*, are also downregulated by PU-PEI-miR145 treatment. These results suggest that PU-PEI-miR145 might suppress the self-renewal and tumor-initiating properties of GBM cells. Notably, miR145 delivery with a combination of radiotherapy (2 Gy) and temozolomide (200 mM) can eliminate tumor formation in vivo.<sup>104</sup> Thus, PU-PEI-miR145 treatment for CSC eradication is a potential therapeutic approach to improve current tumor treatments, especially for tumors that have developed a resistance against conventional therapy. Importantly, polymer-based gene delivery systems are considered to induce low immune responses and are potentially safer than viral-mediated delivery.<sup>104</sup>

## Destruction of CSCs and their niches

Apart from direct targeting of CSCs, various agents are being developed for targeting the microenvironments (niches) of cancer cells.<sup>105</sup> Maintenance of CSC self-renewal involves

cross-talk between CSCs and their supporting stroma or vasculature. New evidence has revealed that CSC cross-talk with their supporting stroma favors tumor progression by promoting cell growth, proliferation, and drug resistance.<sup>106</sup> As such, disruption of the cross-talk between CSCs and their niches is an attractive approach for cancer treatment. An emerging strategy may be employed to design new nanoparticle-based combinatorial therapies for interference of the supportive microenvironmental cross-talk. Currently, many physicochemical treatment methods are being developed to enhance CSC-directed therapy to interfere with CSC niches. Wang et al prepared anti-CD133 monoclonal antibody-conjugated single-walled carbon nanotubes that selectively eradicate CD133+ GMB cells by releasing substantial heat in the nanoenvironment after irradiation with near-infrared laser light.<sup>107</sup> Anti-CD133 monoclonal antibody-conjugated single-walled carbon nanotubes have been demonstrated to be promising heat absorbers, and are used in photothermolysis of malignant cells.<sup>107</sup> After conjugation with an anti-CD133-Phycoerythrin (PE) antibody, nanoparticles retained their photonic features and targeted GBM CD133+ cells. In addition, the in vitro tumorigenic, spheroid body formation, and self-renewal capabilities of GBM CD133+ cells are effectively inhibited because of the localized hyperthermia. Similarly, Burke et al used the efficient heating rates of amide-functionalized multi-walled carbon under irradiation.<sup>108</sup> Stem and bulk breast cancer cells are equally sensitive to nanotube-mediated thermal treatment.<sup>107</sup> The mechanisms of nanotube thermal therapeutic effect are promotion of rapid membrane permeabilization and necrosis of CSCs.

## Telomerase-based therapy of CSCs

Finally, it is noteworthy to mention telomerase-based approaches, as these have potential in nanomedicine-based therapeutics against CSCs. Telomerase is expressed in both bulk tumor cells and CSCs, but has only limited activity in normal tissues and acts as an immortalizing agent. Joseph et al<sup>109</sup> showed that imetelstat (a potent telomerase inhibitor) decreases telomerase activity and suppresses the self-renewal potential of breast CSCs. In addition, imetelstat treatment inhibits tumorigenicity of PANC1 and MDA-MB231 cells in vivo. However, telomerase inhibitors have biopharmaceutical problems such as high hydrophobicity, permeability, and instability. Thus, efficient delivery to target cells and tumors is required. Nanomedicine can overcome their biopharmaceutical shortcomings and ensure that sufficient bioavailability is provided. Although, as far as the authors are aware, no related articles have reported nanopar-

ticles containing a telomerase inhibitor for CSC therapy, such an approach will no doubt be studied in the future.

## Conclusion and future perspectives

New concepts of chemoresistance in cancer have been proposed, which involve the contribution of CSCs to treatment failure. Although the mechanisms responsible for chemotherapy resistance by CSCs have not been clearly identified, overexpression of ABC transporters, a slow rate of self-renewal, and an active DNA repair capacity are all possible pathological mechanisms. In particular, interpreting the cellular heterogeneity in tumors may help to delineate the resistance of cancers to conventional therapies.

Nonetheless, designing nanomedical therapies against CSCs has proven to be complex, possibly because CSCs in the same type of tumor are phenotypically and functionally heterogeneous and because of the nonspecific nature of CSC markers used for targeting. Moreover, CSCs are protected by multiple resistance mechanisms that make them less susceptible to conventional therapies. Nanoparticle-based drugs have the potential to enhance treatments by overcoming chemoresistance or targeting CSCs. We would like to emphasize that elucidating the signaling pathways in CSCs may drive the development of new targeting therapies. Furthermore, Ginsburg and Willard have reported that chemoresistance and treatment effects depend on the distinct patterns of genes associated with stemness/differentiation pathways.<sup>110</sup> Genomic signature (DNA or RNA) differences have recently been exploited to personalize medicine and CSCs, which may facilitate individual-specific nanomedicine and dose selection for better cancer treatment efficacy and patient prognoses.

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## Disclosure

The authors declare no conflicts of interest in this work.

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