Cancer stem cell theory: therapeutic implications for nanomedicine

Ke Wang¹
Xianguo Wu²
Jianwei Wang³
Jian Huang^{1,3}

¹Cancer Institute (Key Laboratory of Cancer Prevention and Intervention, National Ministry of Education; Provincial Key Laboratory of Molecular Biology in Medical Sciences), ²Department of Clinical Laboratory, ³Department 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 are responsible for chemotherapy resistance within a tumor. Several mechanisms of chemoresistance have been proposed, including increased Wnt/ β -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/ β -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).^{1,2} CSCs possess both self-renewal and differentiation capabilities.³ Several signaling pathways are involved in the self-renewal behavior of CSCs, including Wnt/ β -catenin, Notch, and hedgehog signaling, which mediate the resistances against radiotherapy and chemotherapy.⁴ 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.⁵ 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.⁶ Moreover, loaded-drug formulations show excellent tumor cell uptake characteristics, block drug efflux from cancer cells, and reverse the MDR of tumors.^{7,8}

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.^{9,10} 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,¹¹ exhibit increased resistance against conventional radiotherapy and chemotherapy,^{12,13} and may initiate tumors at limiting dilutions in animals.¹⁴ 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.¹⁵ 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).^{16–31} 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.^{26,32} Recently, the CD133+ cell subpopulation was found to harbor brain CSCs.³³ 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+α2β1high, 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.³⁴ 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.³⁵ 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).^{36–42} Understanding the mechanisms of self-renewal in CSCs is of great importance for drug discovery and development. Wnt/β-catenin signaling is one of the key pathways that modulates CSC self-renewal.^{43,44} Activation of Wnt-target genes depends on mediation by β-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*.^{44,45} Beta-catenin protein is degraded by the ubiquitin–proteasome pathway through phosphorylation at Ser33/Ser37/Thr41 by GSK3β.^{46,47} The Wnt/β-catenin pathway is implicated in the maintenance of CSC self-renewal in leukemia, melanoma, and breast, lung, and liver cancers.^{48–52} It has been reported that a high level of β-catenin increases the drug resistance of numerous tumor types,⁵³ indicating that dysregulation

Table 2 Signaling pathways involved in CSCs self-renewal

Major signaling pathways	Cancer	References
Wnt/ β -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- β	Glioblastoma	42

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

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

Notch signaling is another important signaling pathway involved in modulation of CSC self-renewal.^{54–57} 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 4.⁵⁸ The binding of ligands to Notch results in its cleavage by A disintegrin and metalloprotease (ADAM) family members and γ -secretase.⁵⁹ 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- κ B)*.⁶⁰ 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/ β -catenin signaling in diverse cellular situations and the interaction between Notch and Wnt/ β -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.^{61–65} The hedgehog pathway has been identified to be involved in CSC self-renewal and tumorigenicity in human breast cancer.³⁹ Further, a previous report showed that the hedgehog pathway is associated with NF- κ B signaling, indicating that sonic hedgehog might be activated by the transcription factor NF- κ B.⁶⁶

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.^{67,68} Such drug pumps are responsible for protecting cancer cells from damage by cytotoxic chemotherapies via efflux pumping mechanisms.⁶⁹ 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.⁷⁰ The Hoechst efflux assay has successfully identified SP cells in various solid tissues including breast, pancreatic, and liver.^{71–73} Moreover, studies have confirmed that chemoresistant cancer cells contain a higher proportion of SP cells than chemosensitive cells.⁷⁴ 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.⁷⁵ Elevated Oct1 protein expression correlates with elevated CD24–CD44⁺ or ALDH^{hi} 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.⁷⁶ These cells, also termed “label-retaining” cells, can be purified by functional assays.^{77,78} 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.⁷⁹ These observations have been confirmed in CSCs derived from AML and solid tumors.^{80,81} 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.^{82,83} 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.¹³ 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.^{84,85} 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.⁸⁶ ALDH1 is a detoxification enzyme involved in catalyzing the oxidation of acetaldehydes produced from ethanol.⁸⁷ 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,⁸⁸ suggesting that chemoresistant molecules expressed by CSCs will directly affect patient prognoses.

Nanomedical 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 supramolecular 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.⁸⁹ 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.”⁹⁰ 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.^{91,92} 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.^{93,94} Previous studies have demonstrated that cell uptake of drugs is increased using nanovehicles compared with the free drug.⁹⁵ For example, we have developed a novel micelle formulation of oxaliplatin encapsulated in a chitosan vesicle (CSO-SA/OXA micelles).⁹⁶ 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.⁹⁷ 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.⁹⁷

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.⁹⁸ 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.⁹⁸

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.⁹⁹ 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/ β -catenin, hedgehog, and Notch signaling.^{100,101} 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.¹⁰² 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- glycyphenylalanyl-leucylglycyl)-thiazolidine-2-thione (MA-GFLG-TT), followed by conjugation of cyclopamine to glycyphenylalanyl-leucylglycyl side chains. The HPMA copolymer-cyclopamine conjugate binds to cells via the smoothened (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 γ -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.¹⁰³ 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.¹⁰⁴ 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.¹⁰⁴ 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.¹⁰⁴ 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.¹⁰⁴

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.¹⁰⁵ 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.¹⁰⁶ 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+ GBM cells by releasing substantial heat in the nanoenvironment after irradiation with near-infrared laser light.¹⁰⁷ Anti-CD133 monoclonal antibody-conjugated single-walled carbon nanotubes have been demonstrated to be promising heat absorbers, and are used in photothermal lysis of malignant cells.¹⁰⁷ 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.¹⁰⁸ Stem and bulk breast cancer cells are equally sensitive to nanotube-mediated thermal treatment.¹⁰⁷ 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 al¹⁰⁹ 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-

tics 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.¹¹⁰ 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.

Acknowledgment

This study was supported in part by the Zhejiang Provincial Natural Science Foundation of China (grant nos Z2100366, Y2100414, and Y2090386), Science and Technology Bureau of Zhejiang Province (Grant No. 2011C37004), Zhejiang Provincial Program for the Cultivation of High-Level Innovative Health Talents (JH), Stem Cell Engineering and Clinical Translational Medicine (Zhejiang Medical innovation disciplines, JH) and Doctoral Fund of Ministry of Education of China (20100101110124).

Disclosure

The authors declare no conflicts of interest in this work.

References

- Abbott A. Cancer: the root of the problem. *Nature*. 2006;442(7104):742–743.

- Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature*. 2001;414(6859):105–111.
- Gangemi R, Paleari L, Orengo AM, et al. Cancer stem cells: a new paradigm for understanding tumor growth and progression and drug resistance. *Curr Med Chem*. 2009;16(14):1688–1703.
- McCubrey JA, Abrams SL, Stadelman K, et al. Targeting signal transduction pathways to eliminate chemotherapeutic drug resistance and cancer stem cells. *Adv Enzyme Regul*. 2010;50(1):285–307.
- Rich JN, Bao S. Chemotherapy and cancer stem cells. *Cell Stem Cell*. 2007;1(4):353–355.
- Liu Y, Miyoshi H, Nakamura M. Nanomedicine for drug delivery and imaging: a promising avenue for cancer therapy and diagnosis using targeted functional nanoparticles. *Int J Cancer*. 2007;120(12):2527–2537.
- Shapira A, Livney YD, Broxterman HJ, Assaraf YG. Nanomedicine for targeted cancer therapy: towards the overcoming of drug resistance. *Drug Resist Updat*. 2011;14(3):150–163.
- Xu YY, Du YZ, Yuan H, Liu LN, Niu YP, Hu FQ. Improved cytotoxicity and multidrug resistance reversal of chitosan based polymeric micelles encapsulating oxaliplatin. *J Drug Target*. 2011;19(5):344–353.
- Dalerba P, Cho RW, Clarke MF. Cancer stem cells: models and concepts. *Annu Rev Med*. 2007;58:267–284.
- Shackleton M, Quintana E, Fearon ER, Morrison SJ. Heterogeneity in cancer: cancer stem cells versus clonal evolution. *Cell*. 2009;138(5):822–829.
- Ricci-Vitiani L, Lombardi DG, Pilozzi E, et al. Identification and expansion of human colon-cancer-initiating cells. *Nature*. 2007;445(7123):111–115.
- Tan S, Chen JS, Sun LJ, Yao HR. Selective enrichment of hepatocellular cancer stem cells by chemotherapy. *J Int Med Res*. 2009;37(4):1046–1056.
- Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote chemoresistance by preferential activation of the DNA damage response. *Nature*. 2006;444(7120):756–760.
- Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat Rev Cancer*. 2008;8(10):755–768.
- Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med*. 1997;3(7):730–737.
- Fang JS, Deng YW, Li MC, et al. Isolation and identification of brain tumor stem cells from human brain neuroepithelial tumors. *Zhonghua Yi Xue Za Zhi*. 2007;87(5):298–303. Chinese.
- Dontu G, El-Ashry D, Wicha MS. Breast cancer, stem/progenitor cells and the estrogen receptor. *Trends Endocrinol Metab*. 2004;15(5):193–197.
- Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res*. 2005;65(23):10946–10951.
- Dalerba P, Dylla SJ, Park IK, et al. Phenotypic characterization of human colorectal cancer stem cells. *Proc Natl Acad Sci U S A*. 2007;104(24):10158–10163.
- Li C, Lee CJ, Simeone DM. Identification of human pancreatic cancer stem cells. *Methods Mol Biol*. 2009;568:161–173.
- Fang D, Nguyen TK, Leishear K, et al. A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res*. 2005;65(20):9328–9337.
- Bapat SA, Mali AM, Koppikar CB, Kurrey NK. Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. *Cancer Res*. 2005;65(8):3025–3029.
- Eramo A, Lotti F, Sette G, et al. Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ*. 2008;15(3):504–514.
- Yang ZF, Ho DW, Ng MN, et al. Significance of CD90+ cancer stem cells in human liver cancer. *Cancer Cell*. 2008;13(2):153–166.
- Yeung TM, Gandhi SC, Wilding JL, Muschel R, Bodmer WF. Cancer stem cells from colorectal cancer-derived cell lines. *Proc Natl Acad Sci U S A*. 2010;107(8):3722–3727.

26. Huang EH, Hynes MJ, Zhang T, et al. Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colon tumorigenesis. *Cancer Res.* 2009;69(8):3382–3389.
27. Hellsten R, Johansson M, Dahlman A, Sterner O, Bjartell A. Galiellalactone inhibits stem cell-like ALDH-positive prostate cancer cells. *PLoS One.* 2011;6(7):e22118.
28. Hermann PC, Huber SL, Herrler T, et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell.* 2007;1(3):313–323.
29. Huang CP, Tsai MF, Chang TH, et al. ALDH-positive lung cancer stem cells confer resistance to epidermal growth factor receptor tyrosine kinase inhibitors. *Cancer Lett.* 2013;328(1):144–151.
30. Alvero AB, Chen R, Fu HH, et al. Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. *Cell Cycle.* 2009;8(1):158–166.
31. Landen CN Jr, Goodman B, Katre AA, et al. Targeting aldehyde dehydrogenase cancer stem cells in ovarian cancer. *Mol Cancer Ther.* 2010;9(12):3186–3199.
32. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A.* 2003;100(7):3983–3988.
33. Er O. Cancer stem cells in solid tumors. *Onkologie.* 2009;32(10):605–609.
34. Dontu G, Abdallah WM, Foley JM, et al. In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. *Genes Dev.* 2003;17(10):1253–1270.
35. Fan X, Ouyang N, Teng H, Yao H. Isolation and characterization of spheroid cells from the HT29 colon cancer cell line. *Int J Colorectal Dis.* 2011;26(10):1279–1285.
36. Palomero T, Dominguez M, Ferrando AA. The role of the PTEN/AKT Pathway in NOTCH1-induced leukemia. *Cell Cycle.* 2008;7(8):965–970.
37. Korkaya H, Paulson A, Charafe-Jauffret E, et al. Regulation of mammary stem/progenitor cells by PTEN/Akt/beta-catenin signaling. *PLoS Biol.* 2009;7(6):e1000121.
38. Hambardzumyan D, Squatrito M, Carbajal E, Holland EC. Glioma formation, cancer stem cells, and akt signaling. *Stem Cell Rev.* 2008;4(3):203–210.
39. Liu S, Dontu G, Mantle ID, et al. Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. *Cancer Res.* 2006;66(12):6063–6071.
40. Raaphorst FM. Self-renewal of hematopoietic and leukemic stem cells: a central role for the Polycomb-group gene Bmi-1. *Trends Immunol.* 2003;24(10):522–524.
41. Godlewski J, Nowicki MO, Bronisz A, et al. Targeting of the Bmi-1 oncogene/stem cell renewal factor by microRNA-128 inhibits glioma proliferation and self-renewal. *Cancer Res.* 2008;68(22):9125–9130.
42. Peñuelas S, Anido J, Prieto-Sánchez RM, et al. TGF-beta increases glioma-initiating cell self-renewal through the induction of LIF in human glioblastoma. *Cancer Cell.* 2009;15(4):315–327.
43. Kawaguchi-Ihara N, Murohashi I, Nara N, Tohda S. Promotion of the self-renewal capacity of human acute leukemia cells by Wnt3A. *Anticancer Res.* 2008;28(5A):2701–2704.
44. Liu S, Dontu G, Wicha MS. Mammary stem cells, self-renewal pathways, and carcinogenesis. *Breast Cancer Res.* 2005;7(3):86–95.
45. Katoh M. WNT signaling pathway and stem cell signaling network. *Clin Cancer Res.* 2007;13(14):4042–4045.
46. Clevers H. Wnt/beta-catenin signaling in development and disease. *Cell.* 2006;127(3):469–480.
47. Liu C, Li Y, Semenov M, et al. Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. *Cell.* 2002;108(6):837–847.
48. Ysebaert L, Chicanne G, Demur C, et al. Expression of beta-catenin by acute myeloid leukemia cells predicts enhanced clonogenic capacities and poor prognosis. *Leukemia.* 2006;20(7):1211–1216.
49. Li Y, Welm B, Podsypanina K, et al. Evidence that transgenes encoding components of the Wnt signaling pathway preferentially induce mammary cancers from progenitor cells. *Proc Natl Acad Sci U S A.* 2003;100(26):15853–15858.
50. Chien AJ, Moore EC, Lonsdorf AS, et al. Activated Wnt/beta-catenin signaling in melanoma is associated with decreased proliferation in patient tumors and a murine melanoma model. *Proc Natl Acad Sci U S A.* 2009;106(4):1193–1198.
51. Yang W, Yan HX, Chen L, et al. Wnt/beta-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells. *Cancer Res.* 2008;68(11):4287–4295.
52. Teng Y, Wang X, Wang Y, Ma D. Wnt/beta-catenin signaling regulates cancer stem cells in lung cancer A549 cells. *Biochem Biophys Res Commun.* 2010;392(3):373–379.
53. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. *Nat Rev Cancer.* 2005;5(4):275–284.
54. Charafe-Jauffret E, Monville F, Ginestier C, Dontu G, Birnbaum D, Wicha MS. Cancer stem cells in breast: current opinion and future challenges. *Pathobiology.* 2008;75(2):75–84.
55. Gursel DB, Berry N, Boockvar JA. The contribution of Notch signaling to glioblastoma via activation of cancer stem cell self-renewal: the role of the endothelial network. *Neurosurgery.* 2012;70(2):N19–N21.
56. Sikandar SS, Pate KT, Anderson S, et al. NOTCH signaling is required for formation and self-renewal of tumor-initiating cells and for repression of secretory cell differentiation in colon cancer. *Cancer Res.* 2010;70(4):1469–1478.
57. Lee CW, Simin K, Liu Q, et al. A functional Notch-survivin gene signature in basal breast cancer. *Breast Cancer Res.* 2008;10(6):R97.
58. Mumm JS, Kopan R. Notch signaling: from the outside in. *Dev Biol.* 2000;228(2):151–165.
59. Wu JY, Rao Y. Fringe: defining borders by regulating the notch pathway. *Curr Opin Neurobiol.* 1999;9(5):537–543.
60. Borggrefe T, Oswald F. The Notch signaling pathway: transcriptional regulation at Notch target genes. *Cell Mol Life Sci.* 2009;66(10):1631–1646.
61. Clement V, Sanchez P, de Tribolet N, Radovanovic I, Ruiz i Altaba A. HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. *Curr Biol.* 2007;17(2):165–172.
62. Huang FT, Zhuan-Sun YX, Zhuang YY, et al. Inhibition of hedgehog signaling depresses self-renewal of pancreatic cancer stem cells and reverses chemoresistance. *Int J Oncol.* 2012;41(5):1707–1714.
63. Kawaguchi-Ihara N, Okuhashi Y, Itoh M, Murohashi I, Nara N, Tohda S. Promotion of the self-renewal capacity of human leukemia cells by sonic hedgehog protein. *Anticancer Res.* 2011;31(3):781–784.
64. Ferruzzi P, Mennillo F, De Rosa A, et al. In vitro and in vivo characterization of a novel Hedgehog signaling antagonist in human glioblastoma cell lines. *Int J Cancer.* 2012;131(2):E33–E44.
65. Mazumdar T, DeVecchio J, Shi T, Jones J, Agyeman A, Houghton JA. Hedgehog signaling drives cellular survival in human colon carcinoma cells. *Cancer Res.* 2011;71(3):1092–1102.
66. Nakashima H, Nakamura M, Yamaguchi H, et al. Nuclear factor-kappaB contributes to hedgehog signaling pathway activation through sonic hedgehog induction in pancreatic cancer. *Cancer Res.* 2006;66(14):7041–7049.
67. Scharenberg CW, Harkey MA, Torok-Storb B. The ABCG2 transporter is an efficient Hoechst 33342 efflux pump and is preferentially expressed by immature human hematopoietic progenitors. *Blood.* 2002;99(2):507–512.
68. Kim M, Turnquist H, Jackson J, et al. The multidrug resistance transporter ABCG2 (breast cancer resistance protein 1) effluxes Hoechst 33342 and is overexpressed in hematopoietic stem cells. *Clin Cancer Res.* 2002;8(1):22–28.
69. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat Rev Cancer.* 2002;2(1):48–58.

70. Summer R, Kotton DN, Sun X, Ma B, Fitzsimmons K, Fine A. Side population cells and Bcrp1 expression in lung. *Am J Physiol Lung Cell Mol Physiol*. 2003;285(1):L97–L104.
71. Alvi AJ, Clayton H, Joshi C, et al. Functional and molecular characterisation of mammary side population cells. *Breast Cancer Res*. 2003;5(1):R1–R8.
72. Lechner A, Leech CA, Abraham EJ, Nolan AL, Habener JF. Nestin-positive progenitor cells derived from adult human pancreatic islets of Langerhans contain side population (SP) cells defined by expression of the ABCG2 (BCRP1) ATP-binding cassette transporter. *Biochem Biophys Res Commun*. 2002;293(2):670–674.
73. Hussain SZ, Strom SC, Kirby MR, et al. Side population cells derived from adult human liver generate hepatocyte-like cells in vitro. *Dig Dis Sci*. 2005;50(10):1755–1763.
74. Hirschmann-Jax C, Foster AE, Wulf GG, et al. A distinct “side population” of cells with high drug efflux capacity in human tumor cells. *Proc Natl Acad Sci U S A*. 2004;101(39):14228–14233.
75. Maddox J, Shakya A, South S, et al. Transcription factor oct1 is a somatic and cancer stem cell determinant. *PLoS Genet*. 2012;8(11):e1003048.
76. Roth S, Fodde R. Quiescent stem cells in intestinal homeostasis and cancer. *Cell Commun Adhes*. 2011;18(3):33–44.
77. Dembinski JL, Krauss S. Characterization and functional analysis of a slow cycling stem cell-like subpopulation in pancreas adenocarcinoma. *Clin Exp Metastasis*. 2009;26(7):611–623.
78. Horan PK, Melnicoff MJ, Jensen BD, Slezak SE. Fluorescent cell labeling for in vivo and in vitro cell tracking. *Methods Cell Biol*. 1990;33:469–490.
79. Roesch A, Fukunaga-Kalabis M, Schmidt EC, et al. A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. *Cell*. 2010;141(4):583–594.
80. Ishikawa F, Yoshida S, Saito Y, et al. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. *Nat Biotechnol*. 2007;25(11):1315–1321.
81. Teng C, Guo Y, Zhang H, Ding M, Deng H. Identification and characterization of label-retaining cells in mouse pancreas. *Differentiation*. 2007;75(8):702–712.
82. Johannessen TC, Bjerkvig R, Tysnes BB. DNA repair and cancer stem-like cells – potential partners in glioma drug resistance? *Cancer Treat Rev*. 2008;34(6):558–567.
83. Liu G, Yuan X, Zeng Z, et al. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Mol Cancer*. 2006;5:67.
84. Jaecckle KA, Eyre HJ, Townsend JJ, et al. Correlation of tumor O6 methylguanine-DNA methyltransferase levels with survival of malignant astrocytoma patients treated with bis-chloroethylnitrosourea: a Southwest Oncology Group study. *J Clin Oncol*. 1998;16(10):3310–3315.
85. Esteller M, Garcia-Foncillas J, Andion E, et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med*. 2000;343(19):1350–1354.
86. Eyster CE, Rich JN. Survival of the fittest: cancer stem cells in therapeutic resistance and angiogenesis. *J Clin Oncol*. 2008;26(17):2839–2845.
87. Magni M, Shammah S, Schiró R, Mellado W, Dalla-Favera R, Gianni AM. Induction of cyclophosphamide-resistance by aldehyde-dehydrogenase gene transfer. *Blood*. 1996;87(3):1097–1103.
88. Ginestier C, Hur MH, Charafe-Jauffret E, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell*. 2007;1(5):555–567.
89. Cho K, Wang X, Nie S, Chen ZG, Shin DM. Therapeutic nanoparticles for drug delivery in cancer. *Clin Cancer Res*. 2008;14(5):1310–1316.
90. Li SD, Huang L. Pharmacokinetics and biodistribution of nanoparticles. *Mol Pharm*. 2008;5(4):496–504.
91. Chen B, Sun Q, Wang X, et al. Reversal in multidrug resistance by magnetic nanoparticle of Fe₃O₄ loaded with adriamycin and tetrandrine in K562/A02 leukemic cells. *Int J Nanomedicine*. 2008;3(2):277–286.
92. Liu C, Zhao G, Liu J, et al. Novel biodegradable lipid nano complex for siRNA delivery significantly improving the chemosensitivity of human colon cancer stem cells to paclitaxel. *J Control Release*. 2009;140(3):277–283.
93. Gaucher G, Dufresne MH, Sant VP, Kang N, Maysinger D, Leroux JC. Block copolymer micelles: preparation, characterization and application in drug delivery. *J Control Release*. 2005;109(1–3):169–188.
94. Hyung Park J, Kwon S, Lee M, et al. Self-assembled nanoparticles based on glycol chitosan bearing hydrophobic moieties as carriers for doxorubicin: in vivo biodistribution and anti-tumor activity. *Biomaterials*. 2006;27(1):119–126.
95. Dhar S, Reddy EM, Prabhune A, Pokharkar V, Shiras A, Prasad BL. Cytotoxicity of sophorolipid-gellan gum-gold nanoparticle conjugates and their doxorubicin loaded derivatives towards human glioma and human glioma stem cell lines. *Nanoscale*. 2011;3(2):575–580.
96. Wang K, Liu L, Zhang T, et al. Oxaliplatin-incorporated micelles eliminate both cancer stem-like and bulk cell populations in colorectal cancer. *Int J Nanomedicine*. 2011;6:3207–3218.
97. Zhang Y, Zhang H, Wang X, Wang J, 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(2):679–691.
98. Zhou P, Hatzieremias S, Elliott MA, et al. Uptake of synthetic Low Density Lipoprotein by leukemic stem cells – a potential stem cell targeted drug delivery strategy. *J Control Release*. 2010;148(3):380–387.
99. Wang K, Zhang T, Liu L, et al. Novel micelle formulation of curcumin for enhancing antitumor activity and inhibiting colorectal cancer stem cells. *Int J Nanomedicine*. 2012;7:4487–4497.
100. Grudzien P, Lo S, Albain KS, et al. Inhibition of Notch signaling reduces the stem-like population of breast cancer cells and prevents mammosphere formation. *Anticancer Res*. 2010;30(10):3853–3867.
101. Curtin JC, Lorenzi MV. Drug discovery approaches to target Wnt signaling in cancer stem cells. *Oncotarget*. 2010;1(7):563–577.
102. Zhou Y, Yang J, Kopecek J. Selective inhibitory effect of HPMAC copolymer-cyclopamine conjugate on prostate cancer stem cells. *Biomaterials*. 2012;33(6):1863–1872.
103. Mamaeva V, Rosenholm JM, Bate-Eya LT, et al. Mesoporous silica nanoparticles as drug delivery systems for targeted inhibition of Notch signaling in cancer. *Mol Ther*. 2011;19(8):1538–1546.
104. Lo WL, Chien Y, Chiou GY, et al. Nuclear localization signal-enhanced RNA interference of EZH2 and Oct4 in the eradication of head and neck squamous cell carcinoma-derived cancer stem cells. *Biomaterials*. 2012;33(14):3693–3709.
105. Yang ZJ, Wechsler-Reya RJ. Hit ‘em where they live: targeting the cancer stem cell niche. *Cancer Cell*. 2007;11(1):3–5.
106. Zhu X, Zhou X, Lewis MT, Xia L, Wong S. Cancer stem cell, niche and EGFR decide tumor development and treatment response: A bio-computational simulation study. *J Theor Biol*. 2011;269(1):138–149.
107. Wang CH, Chiou SH, Chou CP, Chen YC, Huang YJ, Peng CA. Photothermal ablation of glioblastoma stem-like cells targeted by carbon nanotubes conjugated with CD133 monoclonal antibody. *Nanomedicine*. 2011;7(1):69–79.
108. Burke AR, Singh RN, Carroll DL, et al. The resistance of breast cancer stem cells to conventional hyperthermia and their sensitivity to nanoparticle-mediated photothermal therapy. *Biomaterials*. 2012;33(10):2961–2970.
109. Joseph I, Tressler R, Bassett E, et al. The telomerase inhibitor imetelstat depletes cancer stem cells in breast and pancreatic cancer cell lines. *Cancer Res*. Nov 15, 2010;70(22):9494–9504.
110. Ginsburg GS, Willard HF. Genomic and personalized medicine: foundations and applications. *Transl Res*. 2009;154(6):277–287.

International Journal of Nanomedicine**Dovepress****Publish your work in this journal**

The International Journal of Nanomedicine is an international, peer-reviewed journal focusing on the application of nanotechnology in diagnostics, therapeutics, and drug delivery systems throughout the biomedical field. This journal is indexed on PubMed Central, MedLine, CAS, SciSearch®, Current Contents®/Clinical Medicine,

Journal Citation Reports/Science Edition, EMBase, Scopus and the Elsevier Bibliographic databases. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/international-journal-of-nanomedicine-journal>