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REVIEW ARTICLE

Formulation of nanomedicine as a therapeutic candidate for multidrug-resistant cancer stem cells

Shogo Ozawa*

Department of Pharmacodynamics and Molecular Genetics, School of Pharmacy, Iwate Medical University, Yahaba, Iwate, Japan

Abstract

Cancer stem cells (CSCs) express specific antigens associated with stemness. CSCs derived from solid tumors are resistant to various anticancer agents. Given that the spectrum of drug resistance in CSCs is not well understood, literature searches using various keyword combinations were performed to investigate whether CSCs residing in solid cancers are innately resistant to both lipophilic (e.g., P-glycoprotein substrates) and hydrophilic (e.g., non-P-glycoprotein substrates) substances. Current literature suggests that solid CSCs are more likely to acquire multidrug resistance; that is, they are significantly more likely to resist a wide variety of low-molecular-weight anticancer drugs regardless of whether these drugs are P-glycoprotein substrates. The present review further delineates emerging evidence on the use of nanomedicine to eradicate CSCs.

Keywords: Cancer stem cells; Drug resistance; P-glycoprotein; Solid cancer; Nanomedicine; Eradication of cancer stem cells

***Corresponding author:**
Shogo Ozawa
(sozawa@iwate-med.ac.jp)

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1. Introduction

Cancer stem cells (CSCs) in solid cancers constitute a small subpopulation within a tumor mass. CSCs self-renew, differentiate, contribute to metastasize, and show resistance to anticancer drug therapy.[1,2] Self-renewal of CSCs implies that they can maintain their pool indefinitely. CSCs can differentiate to generate heterogeneous cancer cell lineages. The resultant heterogeneous cells sometimes include cancer non-stem cells. The temporarily emerging cancer non-stem cells may regain stem-like properties due to their plasticity. Meanwhile, general cancer cells are malignant cells that constitute a cancer mass. Thus, all CSCs are cancer cells, but only a portion of cancer cells in a cancer mass are CSCs.

Sloan *et al.*[3] proposed an updated definition of CSCs: Long-term self-renewal must be tested with *in vivo* serial transplantations. CSCs are originally tumor-initiating cells (TICs) of diverse human cancer types. Many laboratories have attempted to reproduce CSC properties *in vitro* to facilitate research on CSCs. For example, Liang *et al.*[4] established a tamoxifen-inducible expression system for the transcription factor Twist, which is involved in the epithelial–mesenchymal transition, using a human breast cancer cell line. Cells activated by tamoxifen, an estrogen receptor ligand, exhibit stem cell characteristics. The authors used tamoxifen-activated breast CSCs and nonactivated

cancer non-stem cells.

The epithelial–mesenchymal transition, which involves glycosphingolipids, is a crucial process in normal embryonic development. CSCs have been found to exhibit an abnormal glycosphingolipid composition, suggesting dysregulated gene expression. Regarding drug resistance, several review articles have described extensive mechanisms underlying chemotherapy resistance (e.g., upregulation of drug efflux transporters, avoidance of apoptosis, and non-coding RNAs).[2]

One of the most serious problems encountered in cancer chemotherapy is that cancer cells develop resistance to anticancer drugs. During the development of resistance, cancer cells may increase active efflux of intracellular drugs. Since the 1970s, extensive evidence has shown that P-glycoprotein functions as a drug efflux pump that uses adenosine triphosphate (ATP) to generate energy for outward drug transport in drug-resistant cells.[5,6]

P-glycoprotein is an ATP-binding cassette (ABC) transporter multidrug resistant protein 1 (MDR1; also known as ATP-binding cassette sub-family B member 1 [ABCB1]). Active drug efflux through CSC membranes also occurs due to the abundant expression of P-glycoprotein/MDR1.[7,8] CSCs are resistant to P-glycoprotein substrate drugs, including doxorubicin, docetaxel, irinotecan, paclitaxel, and etoposide. The signal transducer and activator of transcription 3-mediated expression of *MDR1* is activated together with the activation of the stem cell marker Nanog by the hyaluronan–cluster of differentiation (CD)44 interaction.[9] Thus, MDR1 expression is related to cell stemness.

In contrast, several other laboratories have reported that CSCs are resistant to cisplatin, carboplatin, oxaliplatin, gemcitabine, and 5-fluorouracil, which are significantly less likely to be P-glycoprotein substrates.[10-12] Considering these results from different research laboratories, this study aims to determine whether particular drug-resistant cells reported in a given laboratory exhibit cross-resistance to both P-glycoprotein and non-P-glycoprotein substrate drugs. Thus, a literature search was conducted in PubMed. Search strings combined "doxorubicin" with "5-fluorouracil," "gemcitabine," "cisplatin," "carboplatin," "oxaliplatin," or "irinotecan," together with terms related to drug-resistant CSCs. Another keyword combination was "multidrug-resistant cancer stem cells NOT review," which was used to limit our search to original research articles published as of July 20, 2025. The keyword combination also yielded a considerable number of documents on nanomedicine-mediated eradication of CSCs.

2. Literature search

To identify literature on CSCs' multidrug resistance that was published before July 20, 2025, the following keyword combinations were used in the PubMed database:

(i) "cancer stem cells AND doxorubicin and 5-fluorouracil" (identified 21 papers);
(ii) "cancer stem cells AND cisplatin and 5-fluorouracil" (63 papers);
(iii) "cancer stem cells AND oxaliplatin and 5-fluorouracil" (58 papers);
(iv) "cancer stem cells AND doxorubicin and radiation" (31 papers).
(v) "doxorubicin AND (5-fluorouracil OR gemcitabine OR cisplatin OR irinotecan) drug-resistant cancer stem cells" (209 papers); and
(vi) "multidrug-resistant cancer stem cells NOT review" (868 papers).

The focus of this review article is to identify research articles that examined drug sensitivities to at least two different anticancer drugs between CSCs and cancer non-stem cells. Therefore, literature describing multidrug-resistant CSCs based on concentration–cell survival curves was selected from the 1,250 research articles resulting from the search.

A preliminary literature search for multidrug-resistant CSCs revealed two different methods to establish them: (i) enrichment of CSCs using stem cell markers, such as aldehyde dehydrogenase 1 (ALDH1), CD133,[13] CD44, sex-determining region Y-box 2 (SOX-2), and ABC sub-family G member 2 (ABCG2), and subsequent examination of drug resistance, and (ii) exposure to (an) anticancer drug(s) first and subsequent enrichment of CSCs. In many cases, continuous *in vitro* exposure to a single drug is required to establish drug-resistant CSCs.

The two models were advantageous because papers on isolating CSCs using stem cell markers and those on isolating drug-resistant CSCs via drug exposure were easy to distinguish. However, CSCs obtained by the two methods may not be biologically equivalent.

As the primary focus of the present review article is multidrug resistance in CSCs and a potential nanomedicine formulation to eradicate them, the inclusion criteria were: (i) articles with titles indicating stem cell characteristics and resistance to two or more anti-tumor drugs, and (ii) papers reporting dose–response curves for two or more drugs in both CSCs and cancer non-stem cells. The exclusion criterion was papers reporting resistance to only one anticancer drug, regardless of how sophisticated the mechanisms of resistance were discussed.

3. Cancer stem cells showing resistance to two or more drugs among doxorubicin, 5-fluorouracil, gemcitabine, platinum drugs, and irinotecan (so-called multidrug resistance)

3.1. Enrichment of cancer-stem-cell population based on stem cell markers

Several independent laboratories have succeeded in enriching and characterizing CSC populations. In Table 1, information on CSCs with multidrug resistance is described in chronological order.

Flow cytometry with a phycoerythrin (PE)-conjugated anti-human CD133 antibody was used to separate $CD133^+$ and $CD133^-$ hepatocellular carcinoma stem cells (HCSCs).[14] HCSCs were established from the human hepatocarcinoma cell lines Huh7 and PLC8024. Cell viability of $CD133^+$ cells was higher when exposed to doxorubicin and 5-fluorouracil than that of the $CD133^-$ cells. The degree of resistance of Huh7 cells was approximately 5-fold higher than that of doxorubicin and 2.5-fold higher than that of 5-fluorouracil at the same drug concentration. The drug resistance of $CD133^+$ cells with HCSC properties was associated with preferential expression of survival proteins, including the AKT and B-cell lymphoma 2 pathways,[13] and this association was confirmed using a specific AKT1 inhibitor. A research group recently published a supportive report demonstrating that induction of cancer stemness in hepatocellular carcinoma was achieved through glucose deprivation via dysregulation of AKT signaling.[15]

Cells of the human gastric adenocarcinoma cell line AGS were sorted for $CD44^+$ cells using magnetically activated cell sorting to enrich CSCs.[16] Cellular sensitivity to cisplatin and 5-fluorouracil was compared between $CD44^+$ and $CD44^-$ cells of AGS cells. $CD44^+$ AGS cells were approximately fivefold more resistant to cisplatin and 5-fluorouracil than $CD44^-$ cells. All-trans retinoic acid (1 μM) reduced the degree of resistance to these drugs observed between the $CD44^+$ and $CD44^-$ AGS cells. The investigators recently reported cytotoxic treatments of $CD44^+$ prostate CSCs with curcumin.[17]

The human colorectal cancer line, PLR123, was initially derived from specimens obtained from patients with colorectal cancer.[18] Tumor pieces were minced and implanted into the flanks of NOG mice. Ten human colon cancer xenografts were generated from moderately differentiated colon cancer cells. The xenograft grew rapidly while retaining the ability to reconstitute tumors with epithelial ducts and small budding clusters even after 10 passages in NOG mice. PLR123 cells exhibited approximately 10-fold more resistance to irinotecan (40 μg/mL *vs.* 4 μg/mL) and approximately 8-fold more resistance to oxaliplatin (16 μg/mL *vs.* 2 μg/mL) than the popularly used human colon cancer cell lines HCT116, HT29, SW620, and Colo201. PLR123 cells demonstrated high expression of F-box/WD repeat-containing protein 7 (FBXW7) and consequent c-Myc degradation associated with cancer stemness. Knockdown of FBXW7 using small interfering RNA (siRNA) increased cell sensitivity to these anticancer drugs, strongly suggesting that elevated FBXW7 expression contributes to drug resistance. The investigators also reported more recently that T-lymphoma invasion and metastasis-inducing protein-1 was significantly overexpressed in colon cancer cells that were resistant to 5-fluorouracil, oxaliplatin, and irinotecan.[19]

Peng *et al.*[20] focused on TICs as one of the most important hindrances to limited treatment efficacy, as TICs are a heterogeneous tumor cell subset responsible for tumor recurrence, metastasis, and chemoresistance. Among TIC types, long-term (LT) TICs exhibit the most extensive self-renewal.[19] LT-TICs in colon cancer were isolated from a human colon cancer HT-29 cell line according to the method of isolation of $CD133^+$ and subsequent concentration of $CD44^+$ cells by means of a magnetically activated cell sorting microbead using PE-labeled anti-CD133 antibody and fluorescein isothiocyanate-labeled anti-CD44 antibody. $CD133^+$ and $CD44^+$ cells were highly resistant to oxaliplatin and 5-fluorouracil. The resistance rates to oxaliplatin and 5-fluorouracil were well over fivefold higher than those of $CD133^-$ and $CD44^-$ cells.[20]

Recently, significant progress has been made in cancer therapy by targeting CSC-associated cell-surface molecules, such as CD44 and other macromolecules, using nanomedicine technology. Yao *et al.*[21] first isolated gastric CSCs by sorting $CD44^+$ from AGS cells, a human gastric cancer cell line. The investigators devised novel therapeutic siRNA nanoparticles for dual targeting of CD44 and the glioma-associated oncogene homolog 1 to eradicate CSCs.

Koshkin *et al.*[22] isolated a CSC-like population expressing octamer-binding transcription factor 4 (OCT-4) and SOX-2 stem cell factors using primary human colon adenocarcinoma BSC8 cells. The BSC8 cells were transfected with a reporter plasmid containing the Nanog gene promoter. This reporter assay method used endogenously expressed OCT-4 and SOX-2 transcription factors, which bound to the Nanog promoter in the reporter plasmid. The BSC8 cells with CSC properties were sevenfold more resistant to 5-fluorouracil and fivefold more resistant to oxaliplatin than control BSC8 cells.

Magnetic microbeads conjugated with CD44/CD24

Table 1. Drug resistance spectrum of CSCs enriched by stem cell markers and their properties associated with drug resistance

Cancer/Cell line	Resistant drugs	Properties of CSCs	Reference
Hepatocellular carcinoma/ Huh7, PLC8024 cell lines	Doxorubicin, 5-fluorouracil	$CD133^+$ cells. Highly resistant Expressed AKT and BCL-2 in $CD133^+$ cells	14
Gastric adenocarcinoma/ AGS cell line	Cisplatin, 5-fluorouracil	$CD44^+$ cells. Highly resistant	16
Colorectal cancer/PLR123 cell line	Irinotecan, oxaliplatin	$LGR5^+$ cells. More resistant than HCT116, HT29, SW620, and Colo201. High FBXW7 expression	18
Tumor-initiating colon cancer cells isolated from the HT-29 cell line	Oxaliplatin, 5-fluorouracil	Tumor-initiating cells are isolated as $CD133^+$ and $CD44^+$ cells. Characterized as highly resistant to the two drugs	20
Gastric adenocarcinoma/ AGS cell line	5-Fluorouracil, mitomycin C, epirubicin, docetaxel	$CD44^+$ and $CD44^-$ cells are isolated using flow cytometry	21
Primary colon adenocarcinoma/BSC8 cell line	5-Fluorouracil, oxaliplatin	BSC8 cells are identified as CSCs based on the binding of their endogenous OCT4 and SOX2 proteins to the Nanog promoter	22
Breast cancer/BT549, MCF7 cell lines	Tamoxifen, doxorubicin	CD44/CD24 antibodies are used for CSC detection. Cells expressing the lncRNA ROPM are also associated with CSC	23
Small cell lung carcinoma/ H1048, H1882 cell lines	Doxorubicin, etoposide	Three-dimensional spheroidal cells show stem cell characteristics. CSCs show increased PFKFB3 expression	24
Prostate cancer/22Rv1 cell line	Radiation, docetaxel	Isolated based on high aldehyde dehydrogenase 1 expression ($ALDH^{high}$). $ALDH^{high}$ cells exhibit lower phosphorylated AKT levels and are associated with resistance to ROS-mediated drugs and radiation	25

Abbreviations: BCL-2: B-cell lymphoma 2; CD: Cluster of differentiation; CSC: Cancer stem cell; FBXW7: F-box and WD repeat domain containing 7; LGR5: Leucine-rich repeat-containing G-protein coupled receptor 5; lncRNA: Long non-coding RNA; OCT4: PFKFB3: 6-phosphofructo-2-kinase/ fructose-2,6-biphosphatase 3; Octamer-binding transcription factor 4; ROPM: Regulator of phospholipid metabolism; ROS: Reactive oxygen species; SOX2: SRY-box transcription factor 2.

antibodies were able to obtain cells with stem cell properties from several human breast cancer cell lines, including BT549, Hs578T, MDA231, MDA468, MDA436, MCF7, and T47D.[23] Of these, CSCs and non-CSCs were distinguished by cells expressing or not expressing lncROPM (a long non-coding RNA regulator of phospholipid metabolism), respectively, in the BT549 and MCF7 cell lines. CSCs were approximately 5- to 10-fold more resistant to tamoxifen and doxorubicin than their non-CSC counterparts. lncROPM was highly expressed in breast CSCs, which was associated with abundant production of arachidonic acid and the activation of phosphoinositide 3-kinase (PI3K)/ AKT, Wnt/beta-catenin, and Hippo/Yes-associated protein signaling pathways to contribute to CSC features. Thus, these lncROPM-expressing CSC models shed light on stem cell biology, chemoresistance, and poor prognosis in clinical breast cancer.

Cells with stem cell characteristics in the human small cell lung carcinoma cell lines H1048 and H1882 were enriched as 3D spheroids compared with adherent cells in standard medium.[24] The investigators called these adherent growing cells parental cells. The cell populations with stem cell characteristics expressed ALDH1, CD133, CD44, SOX-2, and ABCG2, which are known stem cell markers. CSCs exhibited increased expression of 6-phosphofructo-2-kinase, a rate-limiting enzyme in glycolysis, which is associated with poor prognosis. Regarding cellular drug sensitivity, CSC-enriched cells were resistant to doxorubicin and etoposide by at least 10-fold, but not to cisplatin, compared with parental cells.

One research group focused on insulin/AKT signaling (a component of the PI3K pathway) and prostate cancer.[25] Prostate CSCs were isolated as ALDH1-high cells ($ALDH^{high}$) from the human prostate cancer 22Rv1

cell line using single-cell sorting. Several ALDH^{high} and low-level ALDH-expressing (ALDH^{low}) clones were established, as high ALDH1 expression is regarded as a putative CSC marker. However, ALDH^{high} cell clones were heterogeneous in terms of tumorigenicity and resistance to radiation and docetaxel. All ALDH1^{high} clones showed lower phosphorylated AKT (Ser473) levels than the ALDH1^{low} clones. The authors explain this intracellular status as follows: When ALDH1 expression is upregulated in CSCs, levels of reactive oxygen species will decrease, and the level of AKT, which is activated by reactive oxygen species, decreases.

Table 1 shows that CSCs isolated or enriched in some laboratories using stem cell markers exhibited resistance to several treatments, including radiation and docetaxel. These resistance profiles differed somewhat across studies. Overall, findings from different research groups suggest that CSCs isolated from various cancers are intrinsically multidrug-resistant. Therefore, if the groups listed in Table 1 had examined a broader range of drugs, these CSCs might be resistant to a significantly wider variety of agents. The summary of isolation/enrichment of CSCs is depicted in Figure 1.

3.2. Induction of cancer stem cells by anticancer drug exposure

Several laboratories have independently performed drug selection experiments and subsequently examined the stemness properties of the drug-selected cancer cells. Therefore, it is important to determine whether all drug-resistant cells generated through selection exhibit CSC characteristics. In this section, information on drug-induced CSCs with multidrug resistance is described.

Lung adenocarcinoma cell lines H460 and H661 were transfected with a green fluorescent protein (GFP) reporter plasmid driven by the CD133 promoter.[26] The transfected cells were isolated based on GFP fluorescence. Seventy percent of the resulting GFP$^+$ cells, which grew more slowly than GFP$^-$ cells in culture medium containing 10% fetal bovine serum (FBS), differentiated and lost GFP expression within two weeks. When GFP$^+$ cells were cultured in ultra-low-adhesion dishes containing stem cell culture medium without 10% FBS, they formed tumor spheres with CSC characteristics. In addition, treating GFP$^+$ cells with low-dose cisplatin (IC_{20}) enriched the CD133$^+$ population and upregulated ABCG2 and ABCB1 expression, thereby increasing cross-resistance to doxorubicin and paclitaxel. This enrichment of CD133$^+$ cells was mediated by activation of Notch signaling,[27,28] a cell-survival pathway, along with mitogen-activated protein kinase, Akt, mammalian target of rapamycin, and nuclear factor-kappa B.

Hepatic CSCs resistant to doxorubicin and 5-fluorouracil were selected through repeated exposure of primary cancer tissue cultures to these drugs.[29] Fresh drug-containing medium was replaced every third day, and cell proliferation was monitored for eight days after empirically optimizing the drug concentrations. The final concentrations were 0.5 μg/mL doxorubicin and 1 μg/mL 5-fluorouracil, which successfully yielded resistance

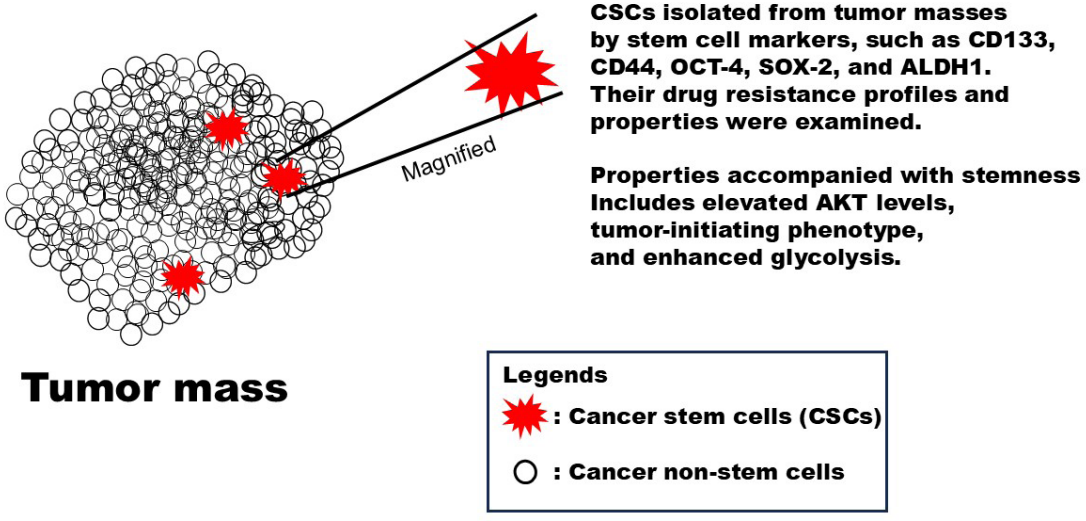


Figure 1. Isolation/enrichment of cancer stem cells and their properties. Image created by the authors using Microsoft PowerPoint.

to two mechanistically distinct drugs. Notably, increased rhodamine 123 efflux and 5-fluorouracil resistance occurred in the same cells during simultaneous treatment with both drugs. The simultaneous treatment might have selected for intrinsically resistant CSCs, consistent with the multidrug resistance described in the previous section. Multidrug resistance induced by selection with two or more drugs is likely to occur in clinical settings, because cancer chemotherapy is generally administered as combination regimens.

One research group treated human colon cancer cell lines with doxorubicin, 5-fluorouracil, oxaliplatin, or irinotecan for 24 h, followed by a 3-day drug-free period.[30] These treatment cycles were repeated six times. These drug treatments induced growth arrest, increased cell size and granularity, led to polyploidization, and augmented the activity of senescence-associated β-galactosidase and the accumulation of P21 and cyclin D1 proteins. In addition, these senescence-inducing agents resulted in delayed repopulation of cancer cells. Furthermore, a subpopulation of senescent colon cancer cells displayed stemness features, including elevated NANOG expression, Hoechst 33342 exclusion, and increased CD24 expression.

Gallego *et al.*[31] established four doxorubicin-resistant bone sarcoma cell lines through stepwise increases in doxorubicin concentration. They reported the following: (i) the 143-B and Saos-2 lines were more doxorubicin-resistant than MG-63 and T-CDS-17#4 cells, and all bone sarcoma cell lines overexpressed P-glycoprotein; (ii) consistent with this, doxorubicin-resistant 143-B and Saos-2 cells expressed significantly higher levels of P-glycoprotein than their parental cells; and (iii) SOX-2 and OCT-4 were upregulated in doxorubicin-resistant 143-B and Saos-2 cells compared with their parental lines. In contrast, SOX-2 and OCT-4 were markedly downregulated in doxorubicin-resistant MG-63 cells compared with the parental line. In T-CDS-17#4 cells, no marked downregulation of SOX-2 and OCT-4 was observed. Taken together, these results indicate that not all cells simultaneously acquire drug resistance and stem cell phenotypes after chemotherapy.

Martins-Neves *et al.*[32] reviewed the induction of stem cell-related phenotypes following treatment with doxorubicin, platinum drugs, DNA-alkylating agents, 5-fluorouracil, gemcitabine, tamoxifen, taxanes, or gefitinib. They noted that some phenotypic changes are shared across doxorubicin-based regimens, platinum-based regimens, alkylating agents, and agents such as 5-fluorouracil and gefitinib. Commonly observed markers included ALDH, CD133, OCT-4, and SOX-2. Because doxorubicin and platinum drugs have considerably different physicochemical properties, these findings suggest that distinct chemotherapeutic agents can converge on similar stemness-associated phenotypic changes.

Cancer stem cells in cancer masses of patients with various solid cancers have multidrug resistance. From a clinical perspective, chemotherapy-induced CSCs emerging after a certain combination of anticancer drugs seem to possess considerably similar characteristics to CSCs isolated from established cancer cells based on stem cell markers, such as CD133, CD44, ALDH, OCT-4, and SOX-2.

4. Eradication of cancer stem cells using various nanomedicine formulations

Solid CSCs may exhibit multidrug resistance to existing low-molecular-weight anticancer drugs. This challenge prompts researchers to develop new therapeutic strategies to target CSCs. A study that described CSC isolation via CD44 positivity also reported a novel therapeutic siRNA nanoparticle.[21] This led to research into new drug therapies, mainly in nanomedicine. Formulations of nanomedicine targeting CSCs are listed in Table 2 by the categories exosome/niosome/liposome, gold/silver, microRNA/siRNA, and nanomedicine hydrogel.

Over the past 5 to 10 years, various nanomedicine formulations targeting solid cancers have been reported, including exosomes, niosomes, liposomes, gold/silver nanoparticles, miRNA/siRNA, and nanomedicine hydrogels. The target solid tumors include osteosarcoma, brain tumors, metastatic breast cancer, oral cancer, and colon cancer (Table 2). The formulations involve NIR irradiation and photothermal therapy with anticancer drugs, which is expected to lead to wider cancer drug therapy.

Table 2. Nanomedicine formulations for cancer therapy, including those targeting CSCs

Formulation	Characteristics of each formulation	Reference
Exosome/Niosome/Liposome	Doxorubicin-encapsulated exosome mimetics demonstrated more potent anti-tumor activity and fewer side effects than free doxorubicin, providing a novel precision medicine for osteosarcoma.	33
	An exosomal delivery system loaded with STAT3-targeted siRNA inhibited tumor proliferation and, *in vivo*, synergistically enhanced temozolomide therapy for glioblastoma.	34
	Epirubicin-loaded niosomal nanoparticle coated with hyaluronic acid successfully decreased breast tumor volume in mice by 28% without side effects on the liver and kidney, as compared with epirubicin alone.	35
	Co-encapsulation of doxorubicin with a short alkyl chain (C_6) ceramide decreased the migratory potential of breast cancer cells, thereby reducing metastasis, although the optimal condition remains to be determined.	36
	Curcumin-loaded niosomes were further decorated with folic acid and polyethylene glycol (PEG-FA@Nio-Cur). PEG-FA@Nio-Cur increased apoptosis-related gene expression and exhibited sustained drug release.	37
	Doxorubicin and ferulic acid-loaded liposomes showed high anti-tumor activity against Lewis lung carcinoma cells and HeLa cells, with no adverse effects on normal cultured cells.	38
Gold/Silver	Gold nanoparticles conjugated to form a CD24–gold nanocomposite, which was more useful for diagnosis and prognosis prediction for salivary gland tumors.	39
	NIR irradiation-potentiated quinacrine–gold hybrid nanoparticles enhanced apoptosis by upregulating caspase-3 and downregulating heat shock protein 70 and Ki-67 in a mouse xenograft model of oral squamous cell carcinoma.	40
	Telaglenastat was loaded into PEGylated gold nanoparticles equipped with a covalently conjugated CD133 aptamer, which was effective against glioblastoma stem cells.	41
	A safe, high-affinity protein-ligand design of Shiga toxin-B (ShTxB) was devised. A gold nanorod was coated with a 35 nm thick rhodamine B isothiocyanate-stained amorphous silica shell (AuNR@SiO_2) for *in cellulo* localization purposes. Finally, ShTxB and AuNR@SiO_2 were conjugated to produce AuNR@SiO_2@ShTxB, which was applied to laser 808 nm-irradiated squamous cell carcinoma head and neck cells. ShTxB showed affinity for the globotriaosylceramide receptor, which is expressed on the surface of preneoplastic and malignant cells in head and neck tumors. The remedy was effective on the isolated cells from patients' head and neck cancer specimens.	42
	PEGylated gold–silver nanoparticles were shown to improve imaging and to sensitize oral cancer KB cells to radiation.	43
	Quinacrine–gold hybrid nanoparticles and NIR radiation retarded the growth of patient-derived primary breast CSCs in a mouse xenograft system by inhibiting tumor growth factor-β-mediated angiogenesis.	44
	SH-PEG-NH(2)-coated gold nanoparticles conjugated to ziyuglycoside I promoted autophagy in hematopoietic stem cells *in vitro* and *in vivo*, suggesting potential application in myelosuppression therapy.	45
	Algae extract and $AgNO_3$ were used to synthesize algae-capped $AgNO_3$ (algae–Ag nanoparticles [AgNPs]). Algae–AgNPs reduced tumor cell migration by downregulating matrix metalloprotease-9 levels. The reduction in breast cancer growth by algae–AgNPs was confirmed using a mouse xenograft model.	46
	Gadolinium chelate-coated gold nanoparticles and conventional X-ray radiation (2 × 5 Gy) reduced the promotion of brain tumor cells with stem-like features.	47
	Surface-engineered hollow gold nanoparticles coated with a mesenchymal stem cell membrane and an MUC-1 aptamer were used to treat a metastatic breast cancer model in mice.	48
	Remote magneto-mechanical actuation of gold-coated magnetic nanoparticles was used to enhance the anti-tumor effects of mitoxantrone on HeLa and adipose-derived stem cells. This combination showed greater effects than mitoxantrone alone.	49

(*Cont'd...*)

Table 2. (*Continued*)

Formulation	Characteristics of each formulation	Reference
MicroRNA/siRNA	Brain-targeted biomimetic siRNA nanoparticles were developed to deliver STAT3 siRNA. These nanoparticles effectively inhibited tumor proliferation and extended median survival time in a mouse xenograft model bearing glioblastoma stem cells.	50
	siRNA targeting peptidyl-prolyl cis-trans isomerase NIMA-interacting 1, loaded into soluble a proliferation-inducing ligand-targeted exosomes, exhibited enhanced anticancer effects by inhibiting epithelial–mesenchymal transition.	51
Nanomedicine hydrogel	Co-encapsulation of salinomycin and a hydrogel within nanomedicine-based lauroyl-gemcitabine lipid nanocapsules reduced glioblastoma and glioma cell viability.	52
	Poly caprolactone-diol (PCL) and PEG were linked together via an ester bond to be a PCL–PEG co-polymer (PCL–PEG). PCL–PEG restricted aggressive regrowth of tumor in an *in vivo* post-surgical glioma xenograft model.	53
	Sequential delivery of doxorubicin followed by quercetin, via polydimethylsiloxane nanoparticles decorated with hyaluronic acid, effectively reduced breast cancer volume by 65% in an SCID mouse xenograft with negligible toxicity.	54
	A pH-responsive oxygen self-sufficient smart nanoplatform (DHCCC), integrating hollow mesoporous silica nanoparticles (HMSNs), chitosan, doxorubicin hydrochloride (DOX), chlorin e6, and catalase (CAT), was fabricated. CAT replenishes oxygen by decomposing hydrogen peroxide, thereby alleviating hypoxia in the tumor microenvironment, which was beneficial for the enhancement of photodynamic therapy (PDT) devised in this study. Controlled DOX release was achieved via the DHCCC–HMSN–DOX system. This synergistic system demonstrated greater anti-tumor efficacy than chemotherapy or PDT alone.	55
	A novel drug–inhibitor conjugate prodrug (PTX-ss-Zos) was prepared from paclitaxel (PTX) and a third-generation P-glycoprotein inhibitor (Zos) linked with a redox-responsive disulfide. PTX-ss-Zos was encapsulated in DSPE–PEG_{2k} micelles to form stable and uniform nanoparticles (PTX-ss-Zos@ DSPE-PEG_{2k} NPs), which exhibited a tumor inhibition rate of 67% in the HeLa/PTX mouse xenograft model.	56

Abbreviations: CSC: Cancer stem cell; DSPE: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine; MUC-1: Mucin-1; NIR: near infrared; PEG: Polyethylene glycol; SCID: Severe combined immunodeficiency; siRNA: Small interfering RNA; STAT3: Signal transducer and activator of transcription 3.

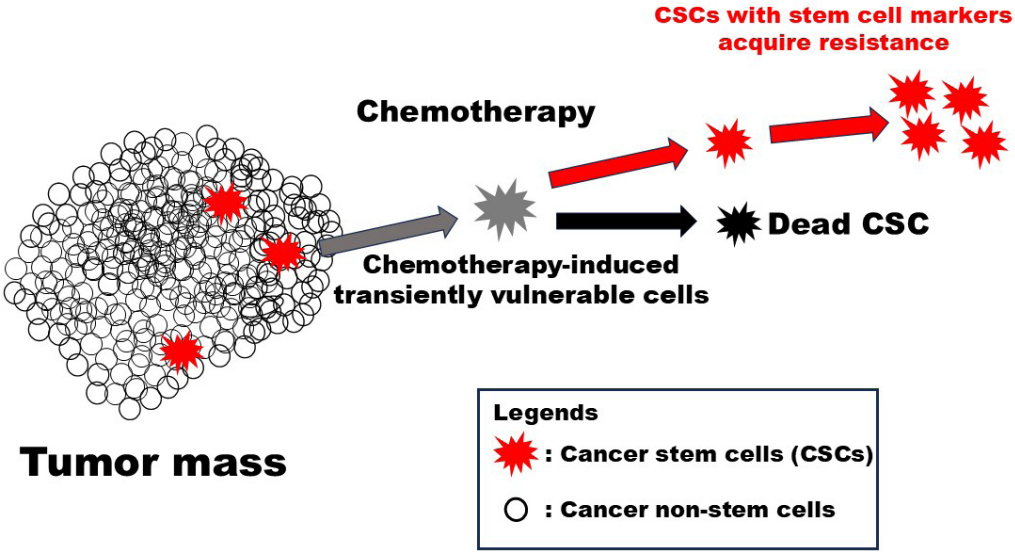


Figure 2. Some cancer stem cells (CSCs) are transiently sensitized by chemotherapy, and upon recovery from chemotherapy-induced cellular damage, they acquire drug resistance. Some CSCs are successfully killed by chemotherapy, whereas others survive, resulting in treatment failure. Image created by the authors using Microsoft PowerPoint.

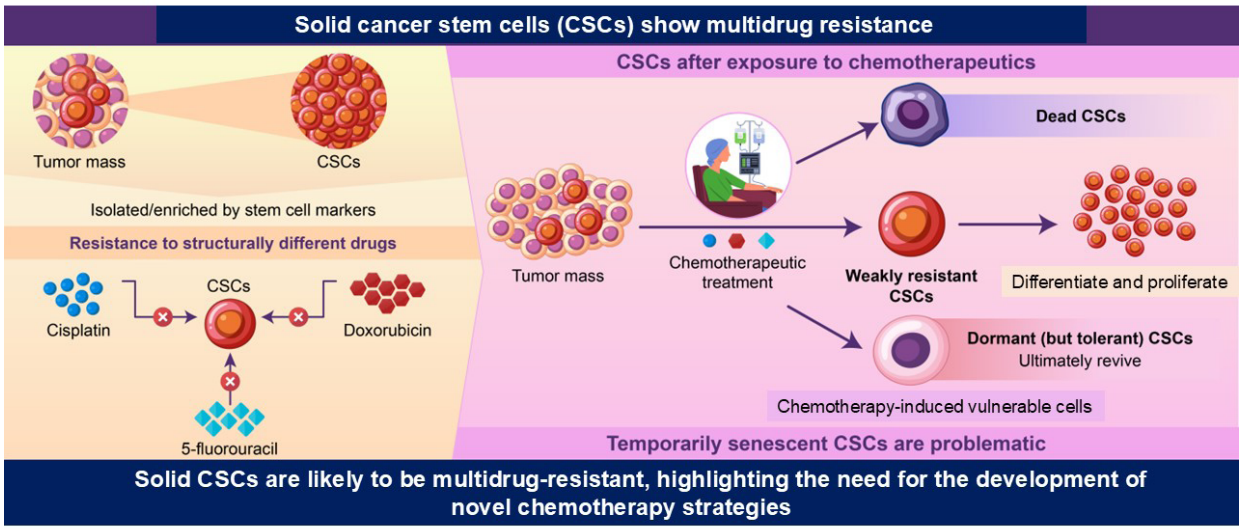


Figure 3. Solid cancer stem cells (CSCs) exhibit multidrug resistance. Consequently, the development of novel chemotherapy strategies, such as potent novel drugs and nanomedicine formulations, is required to effectively target these cells. Image created by the authors using Illustrator (ADOBE).

5. Conclusion

In this review, PubMed searches identified reports showing that CSCs derived from digestive, breast, lung, and prostate tissues were resistant to at least two low-molecular-weight anticancer drugs, including doxorubicin, 5-fluorouracil, cisplatin, irinotecan, oxaliplatin, mitomycin C, epirubicin, docetaxel, tamoxifen, and etoposide. The prostate cancer cell line 22Rv1 is also radiation-resistant. These findings suggest that solid tumors exhibit multidrug resistance, reflecting the intrinsic resistance of CSCs to chemotherapy. This review also summarizes articles describing nanomedicine formulations developed for cancer therapy. Although most of these formulations have so far been evaluated only in cancer cell culture models or in mouse xenografts, their translation into human cancer therapy is anticipated once issues, such as human toxicity and selective drug delivery to tumor tissue, are addressed.

During chemotherapy, some CSCs within the tumor mass are eliminated; however, other CSCs with lower drug sensitivity can survive, differentiate, and repopulate the tumor. In addition, partially damaged CSCs may recover over time. Therefore, new chemotherapeutic regimens that more effectively eradicate cancer cells, including improved nanomedicine approaches, are required. The concluding remarks are summarized in Figure 2, and future perspectives are presented in Figure 3.

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Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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